PLANT GROWTH ANALYSIS OF BORNEAN DIPTEROCARPACEAE SEEDLINGS

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General Introduction

There is a great diversity of organisms in the world and this diversity is hugely important for human well being (Balvanera et al. 2006). In order to protect and utilise this diversity of life we need to understand the mechanisms that maintain diversity. Tropical rainforests harbour high levels of biodiversity and along with that diversity many ecosystem functions. In addition the diversity of tropical rain forests makes them an ideal system to examine how so many ecologically similar species coexist.

Recently the Unified Neutral Theory of Biodiversity (UNTB) caused a stir by recreating patterns of diversity without accounting for species differences (Hubbell 2001). The UNTB is a simple theoretic model of species abundance patterns that does not take into account any biological differences between species. The UNTB's ability to recreate species abundance and diversity patterns surprised many ecologists because the largest amount of what we know about biology is about how species are different. Until this point many ecologists would have expected that models would need to incorporate many species differences in order to come close to recreating patterns of biodiversity and species abundance. This has prompted a re-examination of what we know about niche structure, especially in plant communities.

In contrast to the neutral theory niche partitioning is the process by which two species that exploit the same resource co-exist by separating the resource (Barker et al. 1997). This separation is achieved via different morphological or behavioural adaptations regarding the exploitation of the resource. This separation could occur in time, with species using the same resource during different times of the day, or this separation could occur in space, for example if two species used the same below-

ground resource, but exploited it at different depths controlled by their rooting patterns.

The canopy of the forests of South-east Asia is dominated by trees from the family Dipterocarpaceae (Whitmore 1984). The centre of this diversity is the island of Borneo, with 9 genera of Dipterocarpaceae, 274 species and 20 subspecies (Newman et al 1996). In no other rain forests can such an abundance and diversity of a single family of big trees be found together at a single site (Whitmore 1984). An extremely important phase of the life cycle of these trees is during seedling regeneration (Massey et al. 2006).

Seedling regeneration depends very much on light which is the most limiting and fundamental resource within a forest ecosystem (Thery 2001) . The Gap-size niche partitioning hypothesis has thus been proposed as an important explanation for the coexistence of many closely-related rain forest trees via the utilisation of different light environments (Dalling et al. 2004). Species utilize different environments by trading off traits which are advantageous in particular contrasting environments. These Performance trade-offs are the specialization of traits that allow habitat partitioning in species rich plant communities. Trade-offs take place when the traits that maximise fitness in one situation, hinder the species in another situation (Baraloto et al. 2005).

Plant growth is important to all aspects of ecology - from academic questions understanding the mechanisms of ecosystems such as growth survival trade-offs to more applied questions: for example which species are the most appropriate to begin replanting forests. A recent important theory in ecology that examines the allometric constraints of growth is the West, Brown and Enquist (WBE) theory of metabolic ecology.

West Brown and Enquist theory of metabolic ecology:

The West, Brown and Enquist (WBE) theory of metabolic ecology began with a general model explaining the ¾ power law of metabolic rates through ¼ power scaling of distribution networks (West et al. 1997). Their model predicted the structure and function of a number of biological distribution networks; vertebrate cardiovascular and respiratory systems; plant vascular systems and insect tracheal tubes. They assumed that the terminal tubes (e.g stomata or capillaries) do not vary with body size and argued that all biological distribution networks are predicted to follow quarter power allometric scaling which has evolved through the processes of energy minimization and area preserving branching.

The WBE theory has been shown to explain the evolution of the optimum branching networks amongst vascular plants (West et al. 1999). The WBE theory has since been extended, through a series of high profile papers, into a succession of related predictions about plant physiology; growth, population dynamics and community ecology (Enquist et al. 1998, Enquist et al. 1999, Enquist and Niklas 2001, West et al. 2001, Enquist and Niklas 2002). The theory has attracted great interest because it is based around first principles and makes general predictions about scaling with body size (Coomes 2006).

In the context of this work, it is of particular interest how the WBE framework has also been extended to general growth laws for both plants and animals. Enquist et al. (1999) analysed a 20 year tree diameter growth data-set from a forest in Costa Rica. They found that when they plotted the 20 year diameter$^{2/3}$ against initial diameter$^{2/3}$ - that the slope for the majority of species was indistinguishable from 1. As mass is hypothesised to scale with diameter$^{8/3}$ they concluded that production within species scales as mass$^{3/4}$. While there was considerable between species

variation, this was accounted for by variation between species in wood density. Their model also mathematically accounted for the general decrease in RGR with increasing plant size – that RGR was proportional to Mass $^{-1/4}$.

West et al. (2001) also presented a general theory of ontogenic growth, dealing with animals, based on first principles of energy conservation and allocation. For both their tropical forest dataset and animal data they try to build the model around biological mechanisms rather than the common method of model fitting based primarily on goodness of fit (Enquist et al. 1999, West et al. 2001) . The key is the allocation of energy between maintenance of existing tissue and the production of new biomass.

There has been much controversy over the assumptions of the models that the WBE theory is based around (Coomes 2006). There has been a body of work testing the theory and assumptions of the WBE models, and how well they fit to other data-sets. Coomes et al. (2003) found that accounting for disturbances prevented size-density distributions from following simple scaling relationships. Muller-Landau and colleagues (2006a, 2006b) compared distributions from a large number of tropical forest plots and found that in the majority of cases size distributions differed significantly from the predictions of metabolic ecology. They attributed this to competition for light being more important than the hydraulic restrictions of the theory of metabolic ecology. Reich et al. (2006) also showed that building realistic nutrient dynamics into the model would result in very different model predictions.

Coomes (2006) summarised the challenges to various aspects of the WBE model in particular the WBE models relating to plant community dynamics and size density distributions and highlighted the problems with predictions from these models. We are interested in the scaling rules for growth models (West et al. 2001,

West et al. 2002, West et al. 2004). In this thesis we use one dataset to examine various methods of analysing growth and how this affects our biological understanding of data.

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Summary

This PhD investigates the effect of forest light environments and seed size on the growth of tropical rain forest seedlings. We examine the effect of analytical methods on our biological understanding of data. Rather than compare methods and then continue using one analysis – we completed each analysis through to the biological interpretation of the results. This enabled us to compare how different analytical methods can affect our data interpretation, and show that these are not just statistical nuances but decisions that completely alter our perception of the biology.

In the first chapter we use linear models and traditional metrics of growth such as average relative growth rate (RGR) to investigate the growth of 12 *Dipterocarpaceae* seedlings in three simulated light environments. In addition, we investigated the relationship between growth in each light environment and the seed size of each species.

In the second chapter the same dataset was used to investigate how differences in initial size can change the results. Here we still used linear models and traditional metrics of growth such as average relative growth rate (RGR), but we account for size using covariates. We examine how this can completely change our biological interpretation of the data, and consider the limitations of this analytical technique.

In the third chapter we fit a mechanistic model to our data, that directly corrects for different sized plants. We follow the process and difficulties of this type of analysis. We examine various different functional forms of growth and present results from a few different mechanistic models. We feel mechanistic analysis is the future of understanding plant growth.

The forth general chapter brings together four different plant growth datasets from contrasting plant growth forms and highlights how – regardless of the method

of size correction - size corrected analysis of growth consistently results in different biological interpretation to that of average RGR.

In summary, we believe that size corrected analyses of growth are important to the future of all aspects of ecology. Moreover, as a biological result, this thesis refutes the idea that small seeded species are physiologically adapted to grow faster than larger seeded species.

Zusammenfassung

Diese Dissertation untersucht den Einfluss der Lichtumgebung im Wald und der Samengrösse auf das Wachstum von Sämlingen im tropischen Regenwald. Wir prüfen den Einfluss analytischer Methoden auf unser biologisches Verständnis von Daten. Statt anfangs verschiedene Analysemethoden zu vergleichen um dann mit nur einer weiterzufahren, haben wir jede der verschiedenen Analysemethoden bis hin zur biologischen Interpretation der Resultate verfolgt. Dies ermöglichte uns zu vergleichen wie verschiedene Analysemethoden unsere Interpretation von Daten beeinflussen können und zu zeigen, dass es sich dabei nicht nur um statistische Nuancen handelt, sondern um Entscheidungen die unsere Wahrnehmung der Biologie völlig verändern.

Im ersten Kapitel benutzen wir lineare Modelle und traditionelle Wachstumsmasse, wie die durchschnittliche relative Wachstumsrate (RGR), um das Wachstum von Sämlingen von 12 Arten der Familie *Dipterocarpaceae* in drei simulierten Lichtumgebungen zu untersuchen. Ausserdem untersuchen wir den Zusammenhang zwischen dem Wachstum und der Samengrösse der verschiedenen Arten in jeder Lichtumgebung.

Im zweiten Kapitel wurde derselbe Datensatz verwendet um zu untersuchen wie Grössenunterschiede zu Beginn des Experiments die Resultate verändern können. Dabei verwendeten wir ebenfalls lineare Modelle und traditionelle Wachstumsmasse, wie durchschnittliche relative Wachstumsrate (RGR), jedoch berücksichtigten wir Grössenunterschiede durch die Verwendung von Kovariablen. Wir prüfen wie dies unsere biologische Interpretation der Daten völlig verändern kann und diskutieren die Limitierungen dieser analytischen Technik.

Im dritten Kapitel haben wir ein mechanistisches Modell an unsere Daten angepasst, welches die Grössenunterschiede der Pflanzen direkt berücksichtigt. Wir verfolgen den Prozess und die mit diesem Typ von Analyse verbundenen Schwierigkeiten. Wir prüfen unterschiedliche funktionelle Formen von Wachstum und stellen die Resultate verschiedener mechanistischer Modelle einander gegenüber. Wir denken, dass der mechanistischen Analyse die Zukunft des Verständnisses von Pflanzenwachstum gehört.

Das vierte Kapitel vereint vier verschiedene Datensätze über Pflanzenwachstum und zeigt auf, dass grössenkorrigierte Wachstumsanalyse unabhängig von der verwendeten Methode durchwegs zu einer anderen biologischen Interpretation führt als jene der durchschnittlichen RGR.

Zusammenfassend glauben wir, dass grössenkorrigierte Wachstumsanalysen in Zukunft für alle Aspekte der Ökologie wichtig sind. Als biologisches Resultat widerlegt diese Arbeit ausserdem die Idee, dass Arten mit kleinen Samen physiologisch an schnelleres Wachstum angepasst sind als Arten mit grösseren Samen.

Chapter 1

Growth rate of Dipterocarp seedlings along an experimental light gradient: Conventional analyses of Final Mass, Absolute and Relative Growth Rates

Abstract

The apparent success of the Unified Neutral Theory of Biodiversity (UNTB) in reproducing certain patterns in community ecology has prompted a re-examination of what we know about niche structure, especially in plant communities. The Gap-size niche partitioning hypothesis has been proposed as an explanation for the coexistence of many closely-related rain forest trees. This hypothesis predicts a trade-off, in which species that perform well in a low light environment perform less well in a higher light environment. Seed size is a trait that is thought to be closely related to the growth of plants, especially at the seedling stage, and may explain differences between species growth rates during establishment. We grew 12 species of *Dipterocarpaceae* from seed for one year under different light conditions to test the gap-size niche partitioning hypothesis. Initially we examined the relationship between individual seed mass and seedlings mass for the transition from seed to seedling. We then grew the seedlings in three different light treatments, representing a dark understorey, a small tree-fall gap, and a large tree-fall gap. We found a strong linear relationship between seed-size and 6-week seedlings mass, although larger seeded-species lost proportionally more mass. There was a negative seed-size relative growth rate relationship after 6 weeks. Once the seedlings where growing in the light gradient, most species increased their growth rate sharply between 0.3 % light and 3% light, and began to plateau towards 18% light. Generally the relationship between growth in the mid light and high light treatments was positive; if a species grew well in high light, it also grew well in the mid light treatment. Only two out of the twelve species, *Shorea leprosula* and *Shorea macroptera,* obtained a greater final mass and had a higher absolute growth rate in the mid light treatment, than in the high light treatment. Relative to initial mass, *Shorea leprosula* had almost identical growth in

the mid and high light treatments, whereas *Shorea macroptera* and *Shorea johorensis* both had a higher relative growth rate in the mid light treatment. Species grew the least in the low light treatment, but all survived making growth and survival comparisons impossible.

After one year of growth the largest seeded-species still had the greatest biomass in all three light treatments. Even after accounting for their larger initial size, the larger individuals had grown more in all light treatments. However, per gram of existing biomass, the larger seeded-species are growing slightly less than the smaller-seeded species. In terms of Relative Growth Rates (RGR's) the smaller-seeded species are faster growing compared to the larger-seeded species. During the period of this experiment the size advantage of a greater maternal investment in seed size, enabled the larger seeded individuals to grow faster and remain larger than smaller-seeded species. However, the RGR results suggest that after another year and a half's growth the smaller seeded individuals may catch up and outgrow the larger seeded-species.

Introduction

The apparent success of the Unified Neutral Theory of Biodiversity (UNTB) in reproducing certain patterns in community ecology has prompted a re-examination of what we know about niche structure, especially in plant communities (Hubbell 2001). A key hypothesis, that may explain the coexistence of guilds of similar species in forest systems, is gap-size niche partitioning. Under the gap-size niche partitioning hypothesis, many ecologically similar species can coexist as they have evolved to be preferentially adapted to environmental heterogeneity in light environment. The variation in light environments is determined by the size of canopy gap created by the

fall of a tree. The expectation is that some species perform best in lower light environments, whilst others grow fastest in higher light conditions.

The idea is that a trade-off causes species with a higher relative growth rate (RGR) in the shade to have to lower RGR in a high light environment. This hypothesis predicts shifts (cross overs) in the performance of species between low and high light environments (Sack and Grubb 2001, 2003).

A contrary view holds that if a species grows faster in a high light environment, it will also grow faster in low light (Kitajima 1994, Kitajima and Bolker 2003). Light only plays a role in the maintenance of species richness through a trade-off between growth in the light and survival in the shade. This would predict a negative relationship between growth and survival, rather than rank shifts between light levels. These two hypotheses are often debated in the literature (e.g. Sack and Grubb 2001, Kitajima and Bolker 2003, Sack and Grubb 2003), however it is possible for their to be a trade-off between growth in the light and survival in the shade as well as rank shifts between light intensities.

Species are often divided into groups to permit comparison of growth rates. Forestry classifications results in two groups: “slow growing shade-tolerant” species and “fast growing light demanding” species. Brown and Whitmore (1992) studied the growth of 3 species of dipterocarp existing in the seedling bank for 40 months after creating artificial canopy gaps of different sizes at Danum valley. All species were found to have increased their height growth with increasing gap size. The most important determinant of the size after 40 months was seedling size at gap creation. *Shorea johorensis* had the largest mean size in all but the tiny gaps, but Brown and Whitmore (1992) argue that it’s ultimately one individual that will win the race to fill

the gap, and that the largest individual is more important than the mean size of a species. However if the mean-variance is the same across species then the result will be the same on average. *Hopea nervosa* – a species often grouped by foresters as a slow growing shade-tolerant species – was the species with the largest individual in all of the gap size treatments due to some individuals with a large initial size. Brown and Whitmore (1992) conclude that there was no evidence to support the hypothesis that each species is preferentially adapted to a particular gap size.

Whitmore and Brown (1996) continued measuring their plots for a further three years. After a further year of growth, *Shorea johorensis* began to overtake *Hopea nervosa* in mean height growth, and after 2 more years *Shorea johorensis* was the tallest. Ultimately they found *Shorea johorensis,* the 'light-demanding' species, was the most successful in all but the smallest gaps where *Hopea nervosa,* the 'shade-tolerant' species, could out-grow them (Whitmore and Brown 1996). This data seems to support the hypothesis of gap-size niche partitioning that some species grow fastest in lower light conditions, while others grow fastest in higher light.

In a review of their own and other studies investigating gap size niche partitioning amongst Dipterocarps, Brown et al. (1999) suggested, contrary to their own results, that there was little evidence for niche differentiation to different light environments, on the grounds that competition is highly asymmetric and merely strengthens existing species rankings. Essentially, Brown et al. (1999) purport that available data suggests evidence for growth survival trade-offs and not for gap-size niche partitioning.

Seed size is a key trait for many plant growth forms, and an essential part of the classification of tropical trees into two distinct functional groups: fast-growing pioneers and slower growing climax species (Swaine and Whitmore 1988). Pioneers

often have smaller seeds while climax species have larger seeds. A recent meta-analysis found a negative relationship between seed size and seedling RGR and concluded that seed size is a good surrogate for shade-tolerance of rain-forest tree species (Poorter and Rose 2005). Such an assumption – that there is a growth rate shade-tolerance relationship – is based upon the unsubstantiated belief that there is a ubiquitous growth survival trade off. While Poorter and Rose (2005) found that a relationship between seed-size and growth rate has been shown in many studies at the seedling stage, there is less evidence that this relationship continues throughout the life history of tropical trees.

Dipterocarpaceae are an ideal family of tropical trees to examine the variation within a guild. Nearly all Dipterocarpaceae fit with classification of climax species (Swaine and Whitmore 1988), yet functional traits of this family are extremely diverse. Dipterocarps exhibit seed size variation of a few orders of magnitude and a substantial variation in wood density and SLA (moles, slick et al).

In this study we grew 12 species of dipterocarps from seed for almost 1 year, in order to ask the following questions:

- Do Dipterocarp seedlings change their rank performance in response to light?
- Is seed size a key determinant of this growth variation?
- How do the different traditional metrics of growth compare in their interpretation?

Materials and Methods

Study Site

Danum Valley Field Centre (DVFC) (4°58' N, 118°48' E) is situated within Danum Valley Conservation Area. The 43,800 ha of primary forest is surrounded by the Yayasan Sabah logging concession, located in north-eastern Sabah, Malaysian Borneo. The camp of The Sabah Biodiversity Experiment is based in the Malua forest reserve at 5°5'20"N, 117°38'32"E, which lies around 70 km up the main logging road from DVFC into the Malua forest reserve.

Climate

The Climate at DVFC is aseasonal but subject to occasional drought. On average the field centre receives about 2668 mm of rain every year with a mean annual temperature recorded at the field centre of 26.7°C (Walsh 1996) (Walsh and Newbery 1999). The climate at Malua is believed to be similar and is currently monitored by Philippe Saner and the Malaysian MET Office. (Walsh and Newbery 1999)

Experimental Design

Three Light levels were applied using shade-houses, each of these were replicated five times. The set-up was blocked split-plot design with shadehouse nested within block. Each block of three light treatments was positioned along a north-south line to minimise shading. Seedlings of 12 species were placed in each shade-house. Four individuals of each species were placed in each of the 15 shade-houses.

Shade-houses

The shade-houses were constructed in a large clearing next to the camp of the Sabah Biodiversity experiment, at Malua river. 15 shade houses were constructed (5 x 6 x 5 m) from timber. In order to prevent water-logging and to minimise attacks from snails, the shade houses were raised 0.5 meters from the ground and the seedlings supported by wire mesh. Wire mesh was also attached around the sides of the shade houses to protect the seedlings from deer and elephants.

Measurement of the light environment

70% shade cloth was used to create three different shade treatments, characterised as: (1) a dark forest understorey; (2) a small tree-fall gap; and (3) a large tree-fall gap. As the shading intensity of different brands of shade-cloth varies, and combining layers of shade-cloth can be unpredictable; the light was measured in each of the shade-houses using PAR sensors and data-loggers. One data-logger and PAR sensor was set up in full daylight in the centre of a clearing where there was no shading throughout the day. A second data-logger and PAR sensor was rotated between each of the shade-houses in a block-wise fashion, spending a full day in each shade-house. This 15-day cycle was repeated twice, with a few weeks between repetitions, in order to eliminate effects of weather differences. The clocks of the data-loggers were synchronised, set to take a measurement every 30 seconds, and record average values every 10 minutes. The values for three shading treatments resulted in 18 % ± 0.23, 3 % ± 0.073 and 0.3 % ± 0.024 of full daylight (means ± SEs, n=730) .

Seedlings

During August 2005 a localized fruiting of some species of *Dipterocarpaceae* occurred. The peak of the fruit fall was in the last week of August. Fruiting trees were identified in both primary and secondary forest and the fruit was collected from the ground. Fruit were air dried at the field centre, their wings removed, and seeds weighed. Each seed was numbered and the exact seed weight of each individual seedling recorded. Forest soil was collected, shredded and packed into 4 inch by 9 inch polythene bags. Seeds were placed in the bags, as seedlings began to germinate – the date was recorded and they were placed into the polythene bags of forest soil. The germinating seedlings were then placed under the large nursery of The Sabah Biodiversity Experiment where the shading is approximately similar to the medium light treatment of the shade-houses. Each species was located at slightly different times, and some species germinated faster than others. In order to obtain roughly similar initial experimental conditions, the seedlings were left in this pre-treatment until almost all of them had dropped the remainder of their cotyledons. This process from the start of germination, until seedlings dropped cotyledons, took around 6 weeks.

Species

The species in this study were all those available during the small scale fruiting of 2005. The 12 species available in sufficient numbers were: *Shorea macrophylla* (de Vriese) P.S.Ashton, *Dryobalanops lanceolata* Burck, *Shorea fallax* Meijer, *Shorea parvistipulata* F.Heim, *Shorea falciferoides* Foxw., *Shorea johorensis* Foxw., *Shorea macroptera* Dyer, *Hopea plagata* (Blanco) S.Vidal (giam), *Shorea leprosula* Miq., *Shorea argentifolia* Symington, *Shorea parvifolia* Dyer and *Hopea nervosa*

King.. A thirteenth species was misidentified at the seed stage and later turned out to also be *Hopea nervosa*, so the replication for this species is double that of the others.

Non-destructive measurements

On the 25th of October 2005 four seedlings of each species were placed in each of the 15 shade-houses. A number of non-destructive measures were taken; the leaves of each seedling were counted; two perpendicular measures of diameter were taken at the base of the stem; the height was measured to the apex of the stem.

Initial destructive harvest

At the same time approximately 20 seedlings of each species (range: 11 - 40) were measured in the same way. The roots of these seedlings were carefully washed and each seedling divided into three fractions; stem; roots and leaves. The seedlings were dried in an oven at 80 ˚C for approximately 8 days. A small subset of seedlings was weighed repeatedly to ensure that the biomass was completely dry (Chave 2005).

Allometric relationships

In order to estimate initial biomass of each of the seedlings in the shade-house experiment, allometric relationships were established for each of the species. A separate linear model was fitted for each mass fraction. The final model, that explained 95% of the variation in the data, was:

log(mass) ~ Species * (log(diameter) + height.apex + n.leaves)

Using the non-destructive data from the seedlings at the start of the experiment, and the relationships between the non-destructive measures and biomass, estimated biomass values were predicted for each mass fraction of each seedling.

Measurement of seedlings and destructive harvests

The main shade-house experiment lasted from the 23th of October 2005 until the 13th of August 2006, a total of approximately 293 days growth in the light treatments. After the initial non-destructive measures, and the allometric harvest, there were four separate measures and harvests throughout the year. An attempt was made to evenly space the harvests throughout the year, but they were largely determined by practical constraints. The dates of the harvests were as follows; Initial measure: 23rd-25th of October 2005; 1st Harvest: 7th – 9th December 2005; 2nd Harvest: 18th February and the 22nd of February; 3rd Harvest: 31st of May and the 2nd of June; 4th Harvest: 13th of August 2006. Seedlings were watered daily, and regularly relocated within each shade-house to minimise positioning effects.

During February 2006 there was a period of very heavy rainfall, which resulted in leaching, and many of the seedlings appearing chlorotic. In order to reduce the effects of nutrient deficiency 2.5 grams of Controlled release fertilizer ('Agroblen' - The Scotts Company) was applied immediately after the second harvest.

During the last harvest no seedlings showed any sign of being pot-bound, although the largest seedlings (*Shorea macrophylla*) where beginning to fill their pots.

Methods for statistical analysis

In this chapter we analyse basic metrics of size and growth that are widely used in ecology; total final mass, growth in absolute terms – Absolute Growth rate (AGR); and growth in relative terms to the individuals starting size – Relative Growth Rate (RGR). These metrics were analysed with linear mixed effects models using version 3.1-89 of the nlme library (Pinheiro & Bates) for R 2.7 (R Development Core Team

2007). As the variance varied significantly with species and RGR, we used the varFunc argument in lme to account for differing variances.

Results

Guide to results section

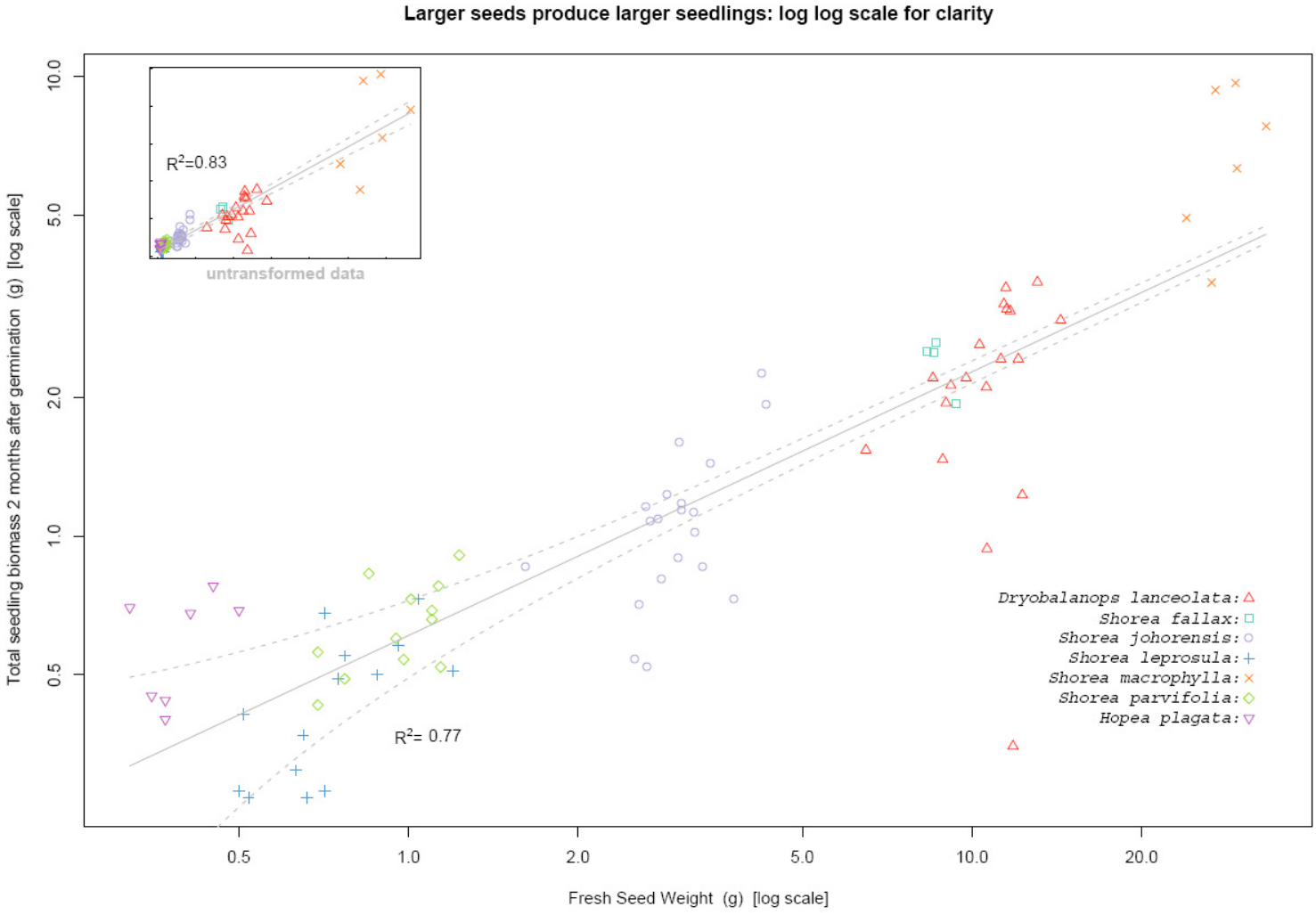
Results of the initial 6 weeks of the experiment are shown in figures 1 - 3, this stage represents the period where the seeds were grown into seedlings. Exact seed weights for individuals are presented, but only for a subset of the species. Figures 1 - 3 start with the most basic metric, final mass, and are followed by absolute growth rate, and relative growth rate. The results from the main shade-house experiment, where the seedlings were grown for almost one year in three different light treatments are shown in figures 4 - 11. Again we start with the total final mass, and progress through AGR and RGR. Results involving seed mass are at the end.

Initial seedling development

Figure 1 shows the relationship between total seedling dry biomass after two months and the fresh seed weight of individual seedlings. The smallest seeded species - *Hopea plagata* - is the only species that has seedling mass larger than the seed mass six weeks after germination. Final biomass is mostly explained by seed size (R^2=0.77, $F_{1, 68}$ = 330.8, P<0.0001), and there is some residual effect of species identity ($F_{6, 68}$ =5.1, P=0.0003). Six weeks after germination it was evident that larger seeds produced larger seedlings (Figure 1), that is, smaller-seeded species do no catch-up or overtake larger seeded ones within this time period. The slope of the relationship between seed size and total seedling mass is shallower than the 1:1 line, indicating that larger seeded individuals lose more mass than smaller seeded individuals.

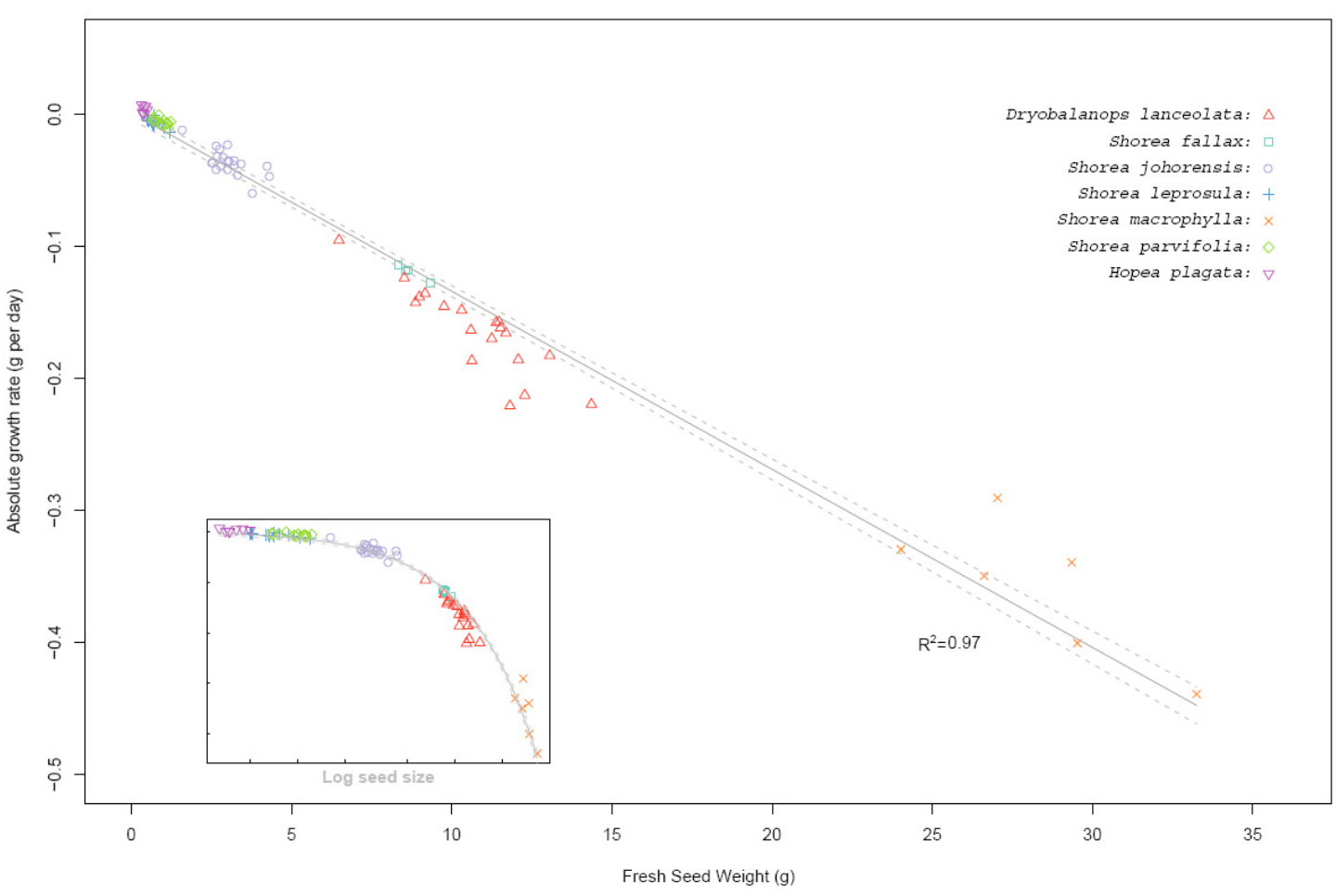
Figure two shows the relationship between Absolute Growth Rate and log seed weight of individual seedlings. In absolute terms the larger seeded individuals loose more mass during the transition from seed to seedling. All but the species with the smallest seeded individuals– *Hopea plagata* – have negative growth rates over the first 6 weeks. Almost all of the variation is explained by individual seed weight ($F_{1, 74}$ = 5544.7, P<0.0001) although there is some effect of species identity ($F_{6, 74}$ = 13.1, P<0.0001).

Relative to their size, the larger seeded individuals have lower growth rates than the smaller species (figure 3). There is an exponential drop in the relative growth rate. As before, the majority of this relationship is explained by seed size ($F_{1, 74}$ = 184.4759, P<0.0001), but there is some effect of species identity ($F_{6, 74}$ = 7.1, P<0.0001). The relationship between relative growth rate and log seed size is linear.


Larger seeds produce larger seedlings: log log scale for clarity
Total seedling biomass 2 months after germination (g) [log scale]
Fresh Seed Weight (g) [log scale]
0.5
1.0
2.0
5.0
10.0
20.0
R²=0.83
untransformed data
R²= 0.77
Dryobalanops lanceolata:
Shorea fallax:
Shorea johorensis:
Shorea leprosula:
Shorea macrophylla:
Shorea parvifolia:
Hopea plagata:

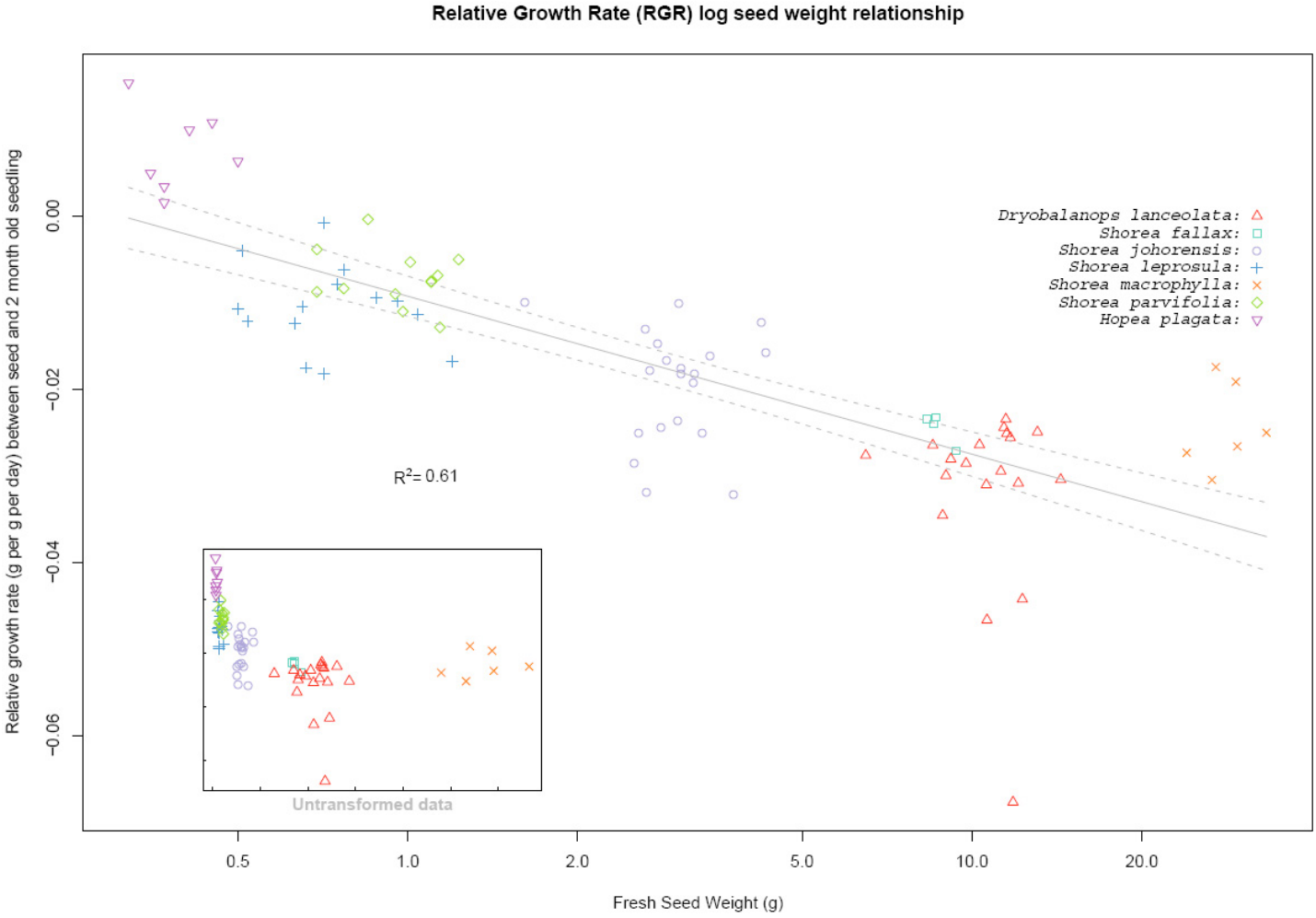
◄ **Figure 1: Larger seeds produce larger seedlings**

The total seedling biomass approximately 2 months after germination for individuals of each species where individual seedling mass and germination date was known. The graph is shown on a log log scale for clarity. The solid grey line shows the regression fit of log seedling mass against log seed weight, the dashed line shows the standard errors of the regression fit. The R^2 value is for the global fit without species identity. The residual effect of species identity is represented by different colours and symbols for each species. The thumbnail in the top left hand corner shows the untransformed data.


Absolute Growth Rate (AGR) between Seed and 2 month old seedling
Absolute growth rate (g per day)
0.0
−0.1
−0.2
−0.3
−0.4
−0.5
0
5
10
15
20
25
30
35
Fresh Seed Weight (g)
Dryobalanops lanceolata:
Shorea fallax:
Shorea johorensis:
Shorea leprosula:
Shorea macrophylla:
Shorea parvifolia:
Hopea plagata:
R²=0.97
Log seed size

◄ **Figure 2: Larger seeded individuals lose more mass**

The Absolute Growth Rate between seed and approximately 2 month old seedling. The grey regression line is shown for the fit without species identity. The grey dashed line shows the standard error for the regression line. The R^2 value is for the linear model without species differences. The Difference in species identity is shown with different colours and symbols for each point. The thumbnail in the bottom left hand corner shows the same data and model with seed size on a log scale because other analysis and authors often present this type of data on a log scale.


Relative Growth Rate (RGR) log seed weight relationship
Relative growth rate (g per g per day) between seed and 2 month old seedling
0.00
−0.02
−0.04
−0.06
0.5
1.0
2.0
5.0
10.0
20.0
Fresh Seed Weight (g)
Dryobalanops lanceolata:
Shorea fallax:
Shorea johorensis:
Shorea leprosula:
Shorea macrophylla:
Shorea parvifolia:
Hopea plagata:
R²= 0.61
Untransformed data

◄ Figure 3: Species with Larger Seeds lose a greater fraction of their mass

The relationship between relative growth rate and log seed weight is linear. The linear model excluding species identity is shown in grey with the solid and dashed line respectively. The R^2 value shown is for the linear model without species identity. Species differences are shown with different symbols and different coloured points. The thumbnail shows the relationship between RGR and seed size on the untransformed scale.

Results - Main experiment

Light treatment has a strong effect on final biomass ($F_{2, 8}$ = 55.7, P<.0001), all of the species increase their biomass as light increases, although this effect is not linear (figure 4). For most species there is a sharp increase in mass from the low light to the mid light treatment, and then the final mass levels off with only a small increase in final biomass in the highest light treatment (figure 4). Species identity has a smaller effect than light on final biomass ($F_{11, 139}$ = 32.2, P<.0001). Generally it is the larger seeded species that have a larger final biomass. There is a marginal difference in the way species respond to light ($F_{22, 139}$ = 1.5, P=0.071). This is likely to be largely driven by *Shorea leprosula* that has a significantly lower biomass in the high light treatment compared to the mid light treatment (figure 4 and figure 5). *Shorea macroptera* grows to a marginally higher final biomass in the mid light treatment, over the high light treatment (figure 4 and figure 5). These two species, which respond quite differently to light compared to the others, largely drive the differences in rank performance seen in Table 1, but there are also some rank changes due to small differences in relative performance.

At the start of the experiment the larger-seeded species had larger seedlings. This seed-size final mass relationship is still very strong after a year's growth (figure 11). The final mass of the seedlings is largely explained by their seed size ($F_{1, 30}$=378.8, P<0.0001), with some effect of the light treatment ($F_{2, 30}$=48.9, P<0.0001). The slope of final mass against seed size is shallower for the low light treatment, than the mid and high light treatment (figure 8, $F_{2, 30}$=27.3, P<0.0001).

Because much of the variation in final mass is explained by seed mass, we can compare the growth in absolute terms using AGR, taking into account initial

differences in seedling size. The confidence intervals on the estimates of AGR are still larger for the larger individuals (figure 6). As with total mass there are few changes in rank performance (Figure 7 & Table 2). However Figure 7 highlights how the lower mass of *Shorea leprosula* in the high light treatment results in it being overtaken in AGR by many of the species. Most of the other rank cross-overs (table 2 & Figure 7) are slight and would be within 95% confidence intervals.

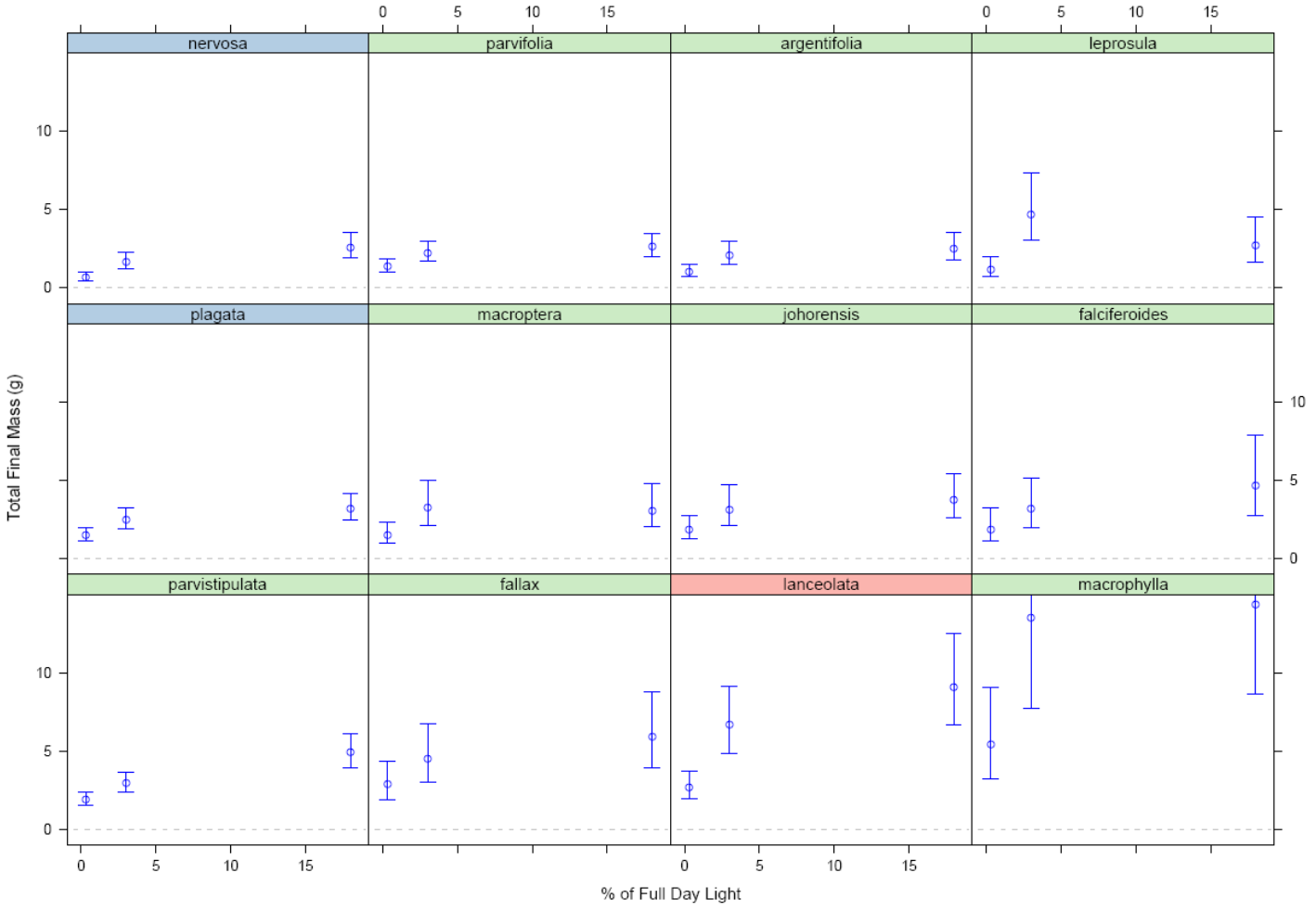
Light affects the AGR ($F_{2, 30} = 56.2$, $P<0.0001$), but the slope of AGR against seed size is significantly different in the three light treatments (Figure 11, $F_{1, 30} = 21.5$, $P<0.0001$), the slope gets steeper with the increase in light and growth. In absolute terms larger-seeded species grow faster. The positive relationship with seed size is still strong (Figure 11, $F_{1, 30} = 146.1$, $P<0.0001$) in all of the light treatments. Despite removing the effect of initial biomass, and taking account of the difference in starting mass, the picture painted by the AGR is similar to analysing final mass.

Figure 8 shows RGR - the growth of the different species relative to their starting mass. This measure of efficiency uses units of grams per gram per day. Here the relationship between growth and light treatment is similar to before, with a sharp increase in growth from low to mid light, and a small increase, or levelling off between mid and high light treatments ($F_{2, 8} = 143.1$, $P<0.0001$). There are differences in mean species relative growth rates ($F_{11, 139} = 9.0$, $P<0.0001$), and a small difference in the way species respond to light ($F_{22, 139} = 1.7$, $P<0.0001$). *Hopea nervosa,* the smallest-seeded seedling now has one of the highest growth rates per gram of mass, whereas *Shorea macrophylla,* the largest-seeded seedling has one of the lowest growth rates per gram.

Figure 9 highlights how the different responses to light shown in figure 8 can lead to changes in rank performance. As the irradiance is increased *Shorea leprosula* does not increase its RGR, resulting in *Hopea nervosa* being the fastest growing species. *Dryobalanops lanceolata* increases its RGR rapidly in response to light, outranking seven other species. *Shorea johorensis* decreases its RGR in response to light enabling three other species to out rank it.

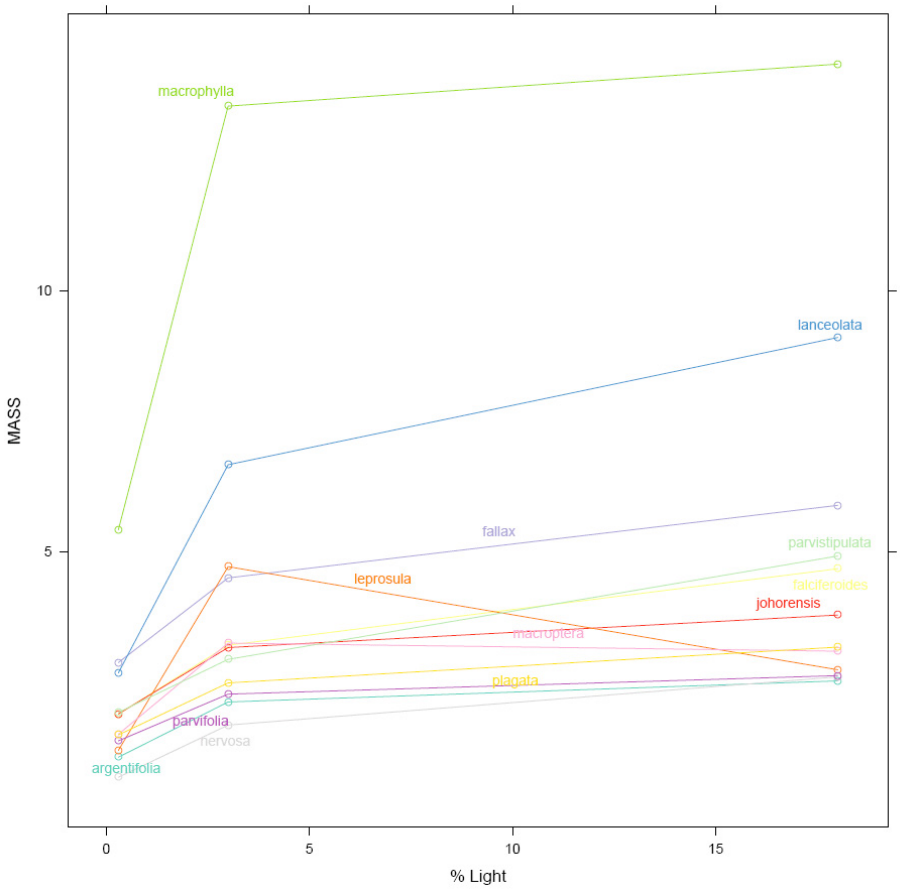
The relationship between Relative growth rate and seed size for each light treatment is shown in the first row of panels in figure 11. There is a negative relationship between seed size and RGR, this relationship is steepest and strongest in the middle light treatment.

Figure 10 summarizes the differences in final mass, absolute growth rate and relative growth rate between mid and high light seen in the last few graphs. Generally if a species grows well in the mid light treatment, then they also grow well in the high light treatment. Most of the species are slightly above the 1:1 line indicating that they grow slightly better in the higher light. *Shorea leprosula* achieves a higher final biomass, and has a faster AGR in the mid light treatment, but its RGR is very slightly higher in the high light treatment. *Shorea macroptera* performs best in the mid light treatment regardless of the metric. Relative to initial size *Shorea johorensis* performs best in the mid light treatment, but it's final mass and AGR are highest in the high light treatment.


nervosa
parvifolia
argentifolia
leprosula
plagata
macroptera
johorensis
falciferoides
parvistipulata
fallax
lanceolata
macrophylla
Total Final Mass (g)
% of Full Day Light
0
5
10
15

◄Figure 4

Total final mass for each seedling after almost 1 year of growth in each of the three light treatments. The points are estimates taken directly from the Linear Mixed Effects Model, the error bars are 95% confidence intervals. The panels are ordered according to seed weight, with the smallest in the top left (*Hopea nervosa*), increasing in size from left to right, from top to bottom, the largest seeded species being at the bottom right (*Shorea macrophylla*). The strips are coloured according to Genus identity: *Shorea's* have a green strip, *Hopea's* a blue strip and *Dryobalanops lanceolata* a read strip. The upper limits of the confidence intervals for *Shorea macrophylla* extend to 23.8 g in 3% light and 23.8 g in 18% light.



Total Final Mass Rank Order

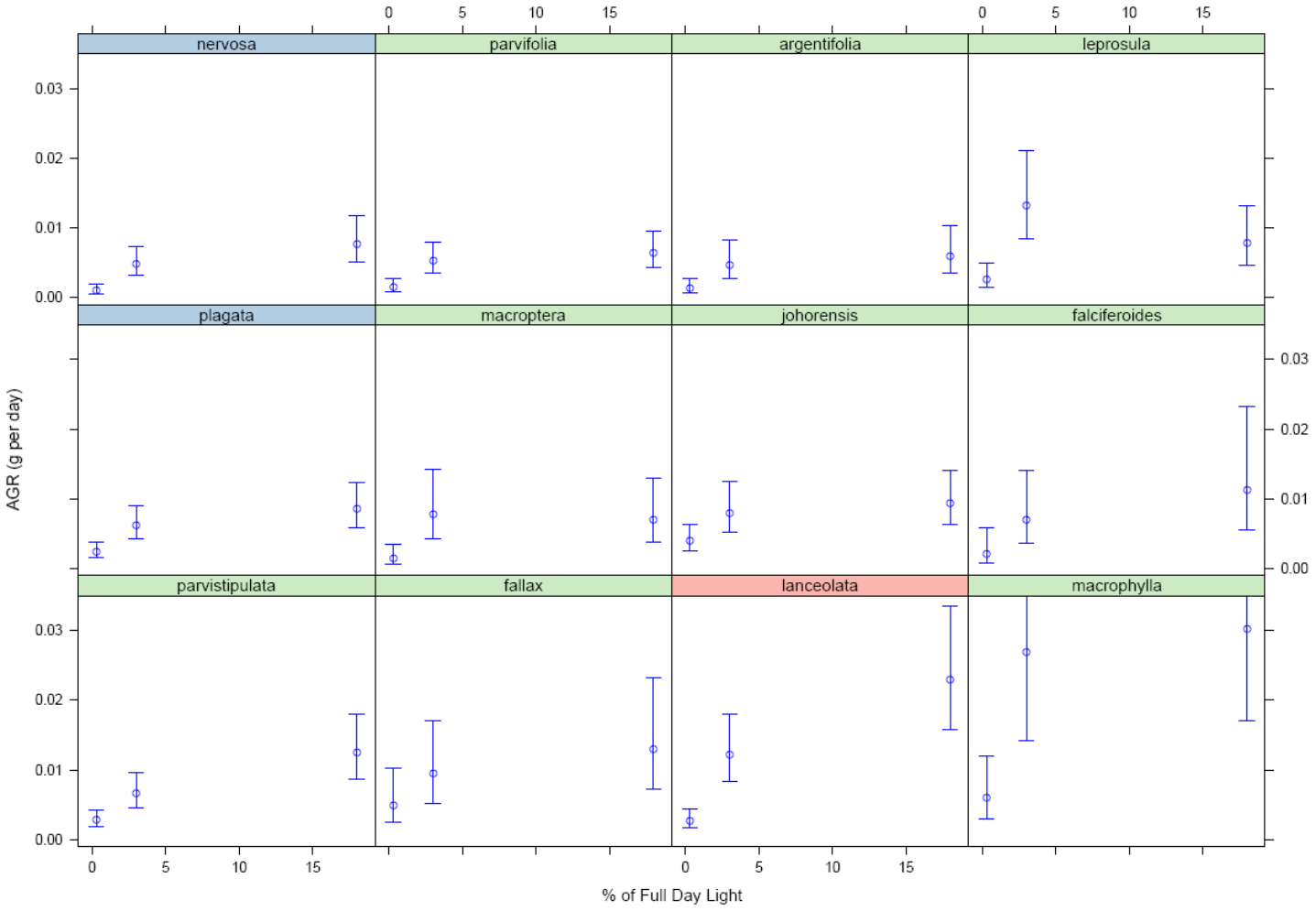
	Light Treatment		
Species:	low	mid	high
Shorea macrophylla:	1	1	1
Shorea fallax:	2	4	3
Dryobalanops lanceolata:	3	2	2
Shorea parvistipulata:	4	8	4
Shorea johorensis:	5	7	6
Shorea falciferoides:	6	6	5
Shorea macroptera:	7	5	8
Hopea plagata:	8	9	7
Shorea parvifolia:	9	10	10
Shorea leprosula:	10	3	9
Shorea argentifolia:	11	11	12
Hopea nervosa:	12	12	11

◀ **Figure** 5: **Cross-overs in total mass**

The same estimates of final mass as in figure 4 for each of the light treatments, each species is shown on one panel, in a different colour to highlight the rank cross-overs. Lines are drawn between the estimates for each light treatment to give an idea of where the cross over takes place. There are no cross overs with *Shorea macrophylla* which clearly has the highest mass in all light treatments.

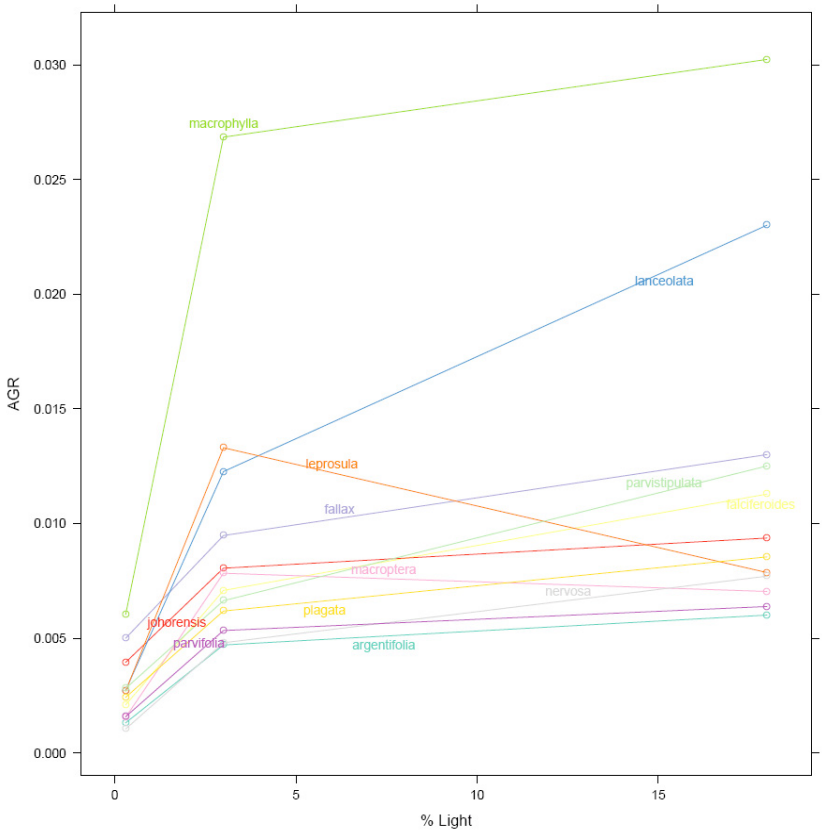
◀ **Table 1: The rank order for each species**

Species are ordered according to their rank in the low light treatment. The numbers indicate how each species changes rank as the light treatment changes


nervosa
parvifolia
argentifolia
leprosula
plagata
macroptera
johorensis
falciferoides
parvistipulata
fallax
lanceolata
macrophylla
AGR (g per day)
% of Full Day Light
0.00
0.01
0.02
0.03
0
5
10
15

◄ Figure 6

Absolute Growth Rate for each seedling after almost 1 year of growth in each of the three light treatments. As before, the points are estimates taken directly from the Linear Mixed Effects Model, the error bars are 95% confidence intervals. The panels are ordered according to seed weight, with the smallest in the top left (*Hopea nervosa*), increasing in size from left to right, from top to bottom, the largest seeded species being at the bottom right (*Shorea macrophylla*). The strips are coloured according to Genus identity: *Shorea's* have a green strip, *Hopea's* a blue strip and *Dryobalanops lanceolata* a red strip. The upper limits of the confidence intervals for *Shorea macrophylla* extend to 0.051 in 3% light and 0.055 in 18% light.



Absolute Growth Rate Rank Order

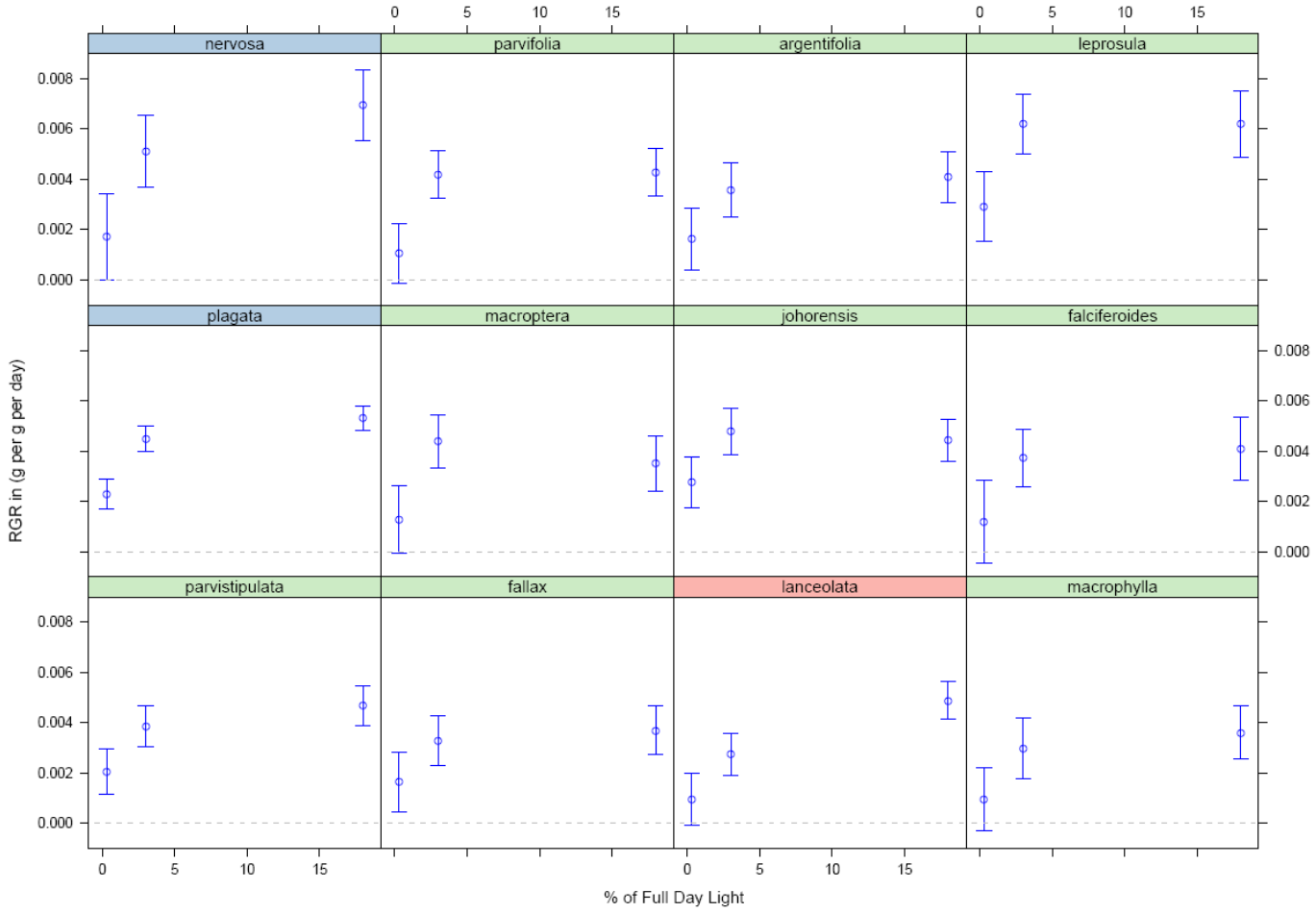
Species:	low	mid	high
Shorea macrophylla:	1	1	1
Shorea fallax:	2	4	3
Shorea johorensis:	3	5	6
Shorea parvistipulata:	4	8	4
Dryobalanops lanceolata:	5	3	2
Shorea leprosula:	6	2	8
Hopea plagata:	7	9	7
Shorea falciferoides:	8	7	5
Shorea parvifolia:	9	10	11
Shorea macroptera:	10	6	10
Shorea argentifolia:	11	12	12
Hopea nervosa:	12	11	9

◄Figure 7: Cross-overs in Absolute Growth Rate

Estimates of Absolute Growth Rate for each of the light treatments, with each species shown on one panel to highlight the rank cross-overs. There are no cross overs with *Shorea macrophylla* which clearly has the highest AGR in all light treatments.

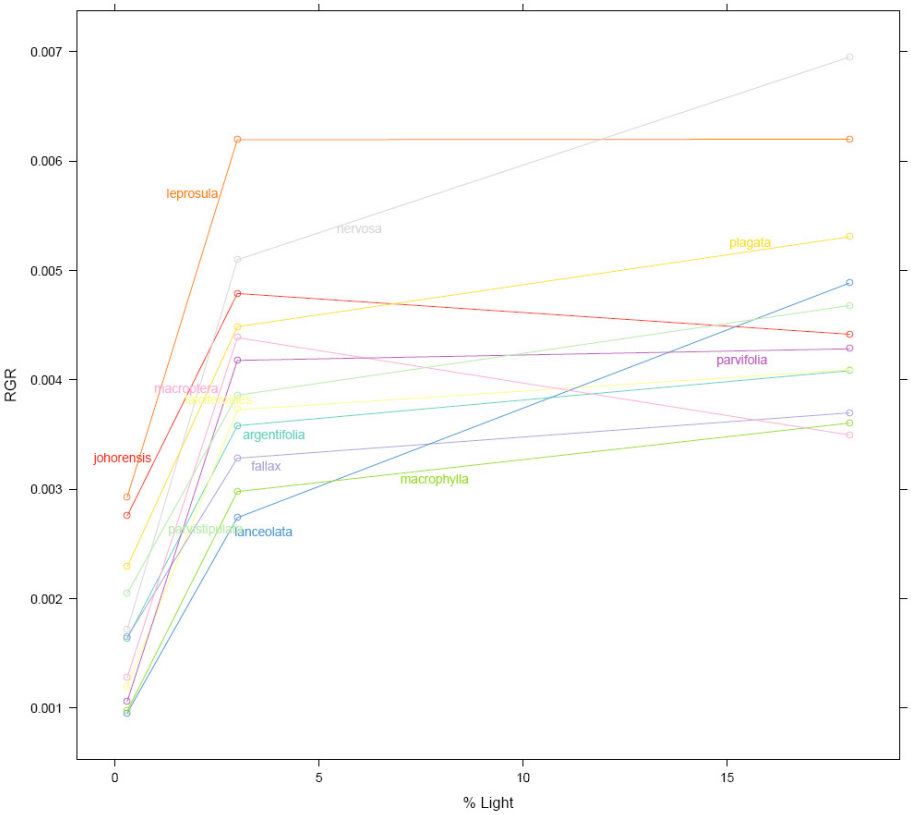
◄Table 2: The rank order in AGR for each species

Species are ordered according to their rank in the low light treatment. The numbers indicate how each species changes rank as the light treatment changes


nervosa
parvifolia
argentifolia
leprosula
plagata
macroptera
johorensis
falciferoides
parvistipulata
fallax
lanceolata
macrophylla
RGR in (g per g per day)
% of Full Day Light
0.000
0.002
0.004
0.006
0.008
0
5
10
15

◄ Figure 8

Relative Growth Rate for each seedling after almost 1 year of growth in each of the three light treatments. As before, the points are estimates taken directly from the Linear Mixed Effects Model, the error bars are 95% confidence intervals. The dashed grey line highlights where the 95% confidence intervals cross zero. The panels are ordered according to seed weight, with the smallest in the top left (*Hopea nervosa*), increasing in size from left to right, from top to bottom, the largest seeded species being at the bottom right (*Shorea macrophylla*). The strips are coloured according to Genus identity: *Shorea's* have a green strip, *Hopea's* a blue strip and *Dryobalanops lanceolata* a red strip.



Relative Growth Rate Rank Order

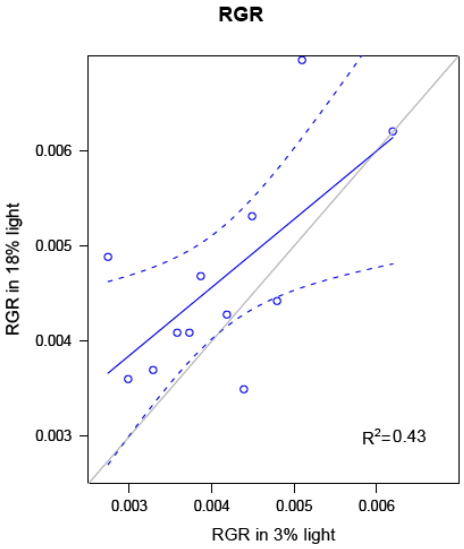
Species	low	mid	high
Shorea leprosula:	1	1	2
Shorea johorensis:	2	3	6
Hopea plagata:	3	4	3
Shorea parvistipulata:	4	7	5
Hopea nervosa:	5	2	1
Shorea fallax:	6	10	10
Shorea argentifolia:	7	9	9
Shorea macroptera:	8	5	12
Shorea falciferoides:	9	8	8
Shorea parvifolia:	10	6	7
Shorea macrophylla:	11	11	11
Dryobalanops lanceolata:	12	12	4

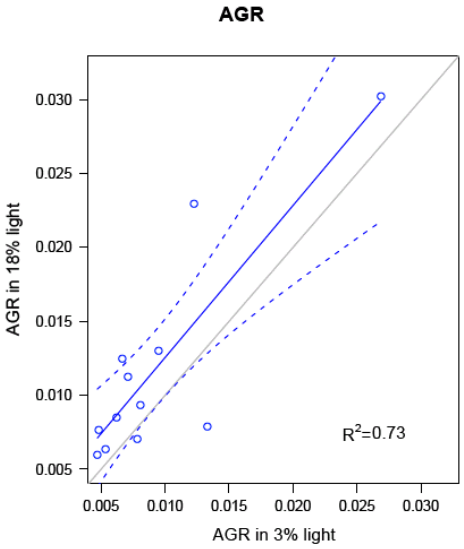
◄Figure 9: cross overs in Relative Growth Rate

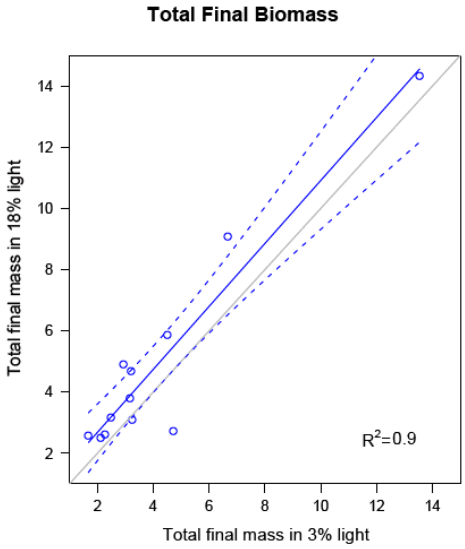
Estimates of Relative Growth Rate for each of the light treatments, with each species shown on one panel to highlight the rank cross-overs.

◄Table 3: The rank order in RGR for each species

Species are ordered according to their rank in the low light treatment. The numbers indicate how each species changes rank as the light treatment changes

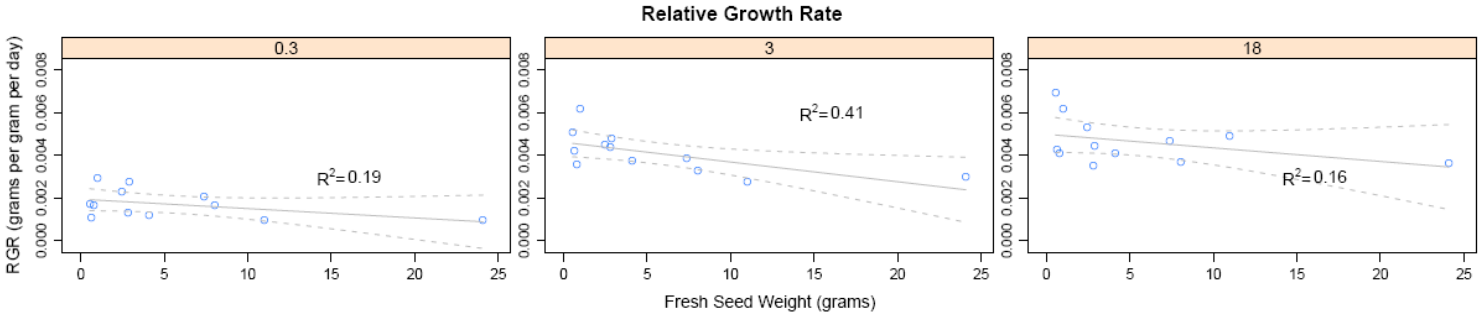

RGR
RGR in 18% light
RGR in 3% light
0.003
0.004
0.005
0.006
R²=0.43

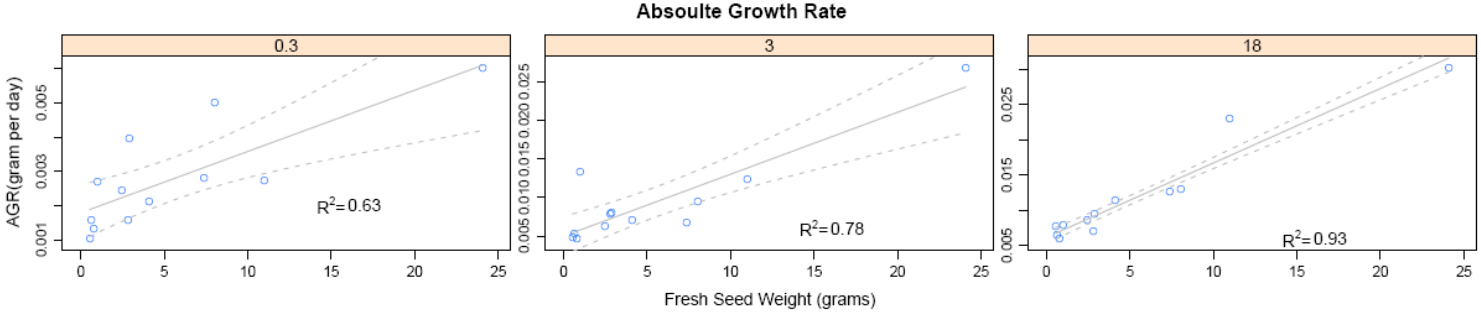

AGR
AGR in 18% light
AGR in 3% light
0.005
0.010
0.015
0.020
0.025
0.030
R²=0.73

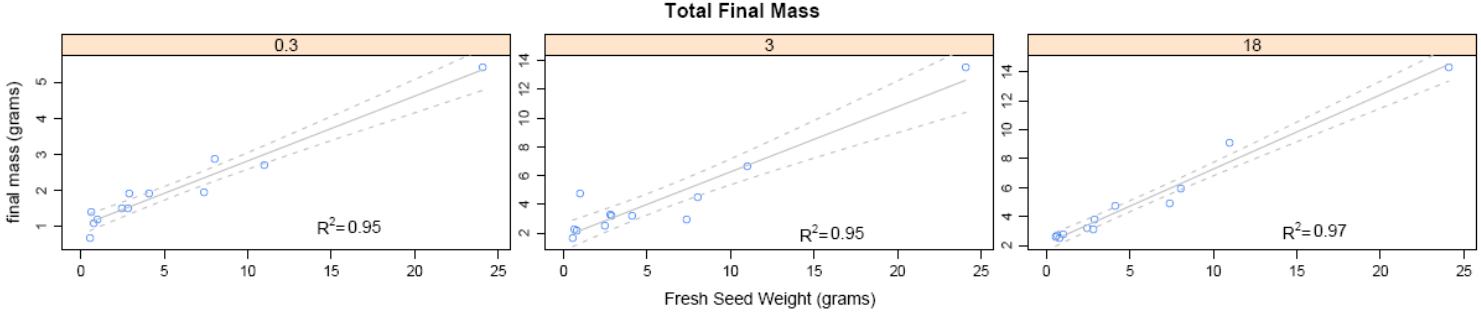

Total Final Biomass
Total final mass in 18% light
Total final mass in 3% light
2
4
6
8
10
12
14
R²=0.9

◄ **Figure 10**

One panel for each of the metrics; from left to right; Relative Growth Rate, Absolute Growth Rate, Total Final Biomass. Each panel shows growth, or size, in the high light against growth, or size, in the mid light treatment with one point for each species. The 1:1 line in each panel highlights where a species performs better in one of the light treatments. Points occurring below the line indicate seedlings that have performed better in the mid light treatment, while points above the line represent seedlings that have performed better in the high light treatment.


Relative Growth Rate
0.3
3
18
RGR (grams per gram per day)
R^2=0.19
R^2=0.41
R^2=0.16
Fresh Seed Weight (grams)


Absoulte Growth Rate
0.3
3
18
AGR(gram per day)
R^2=0.63
R^2=0.78
R^2=0.93
Fresh Seed Weight (grams)


Total Final Mass
0.3
3
18
final mass (grams)
R^2=0.95
R^2=0.95
R^2=0.97
Fresh Seed Weight (grams)

◄ Figure 11: Seed Size Growth relationships in each light treatment

There is one row for each metric, Relative Growth Rate, Absolute Growth Rate and Total Final Mass from the top. There is one panel for each light treatment, from left to right; 0.3%, 3% and 18% light. Each panel has one point per species, and as the x axis are the same, the points can be lined up vertically to more easily compare the AGR, RGR and Final Mass values.

Discussion

Transition from seed to seedling

We found a strong positive linear relationship between seed-size and 6-week old seedling mass (Figure 1), although larger seeded-species lost proportionally more mass (Figure 3). This is in agreement with the results of Cornelissen (1996) who also found that larger seeded species of trees from the British Isles initially produce larger seedlings. Cornelissen (1996) defined this initial stage as when the seedling had opened, but not necessarily expanded its first leaf. We waited until all leaves were expanded, and cotyledons excised, this may have inadvertently allowed time for the smaller seeded species to begin growing, while the larger seeded species were still in the transition stage. However Cornelissen (1996) also found a strong linear relationship between initial seedling biomass, and biomass of seedlings after 3 weeks. Hence we would expect the same relationship to be found if a different harvest time was chosen for the initial period, suggesting that harvest date does not have an influence on this relationship.

The 1:1 line on Figure 1, highlights that *Hopea plagata* is gaining weight from fresh seed to seedling biomass in the 6 week period. It may be that if it had been possible to use dry seed mass, that the next two smallest species *Shorea leprosula* and *Shorea parvifolia,* would also be above this line, and gaining biomass in this period. Nevertheless, it is clear that the smaller seeded species are loosing proportionally less mass in this seed to seedling period and are therefore more efficient. If a 1:1 line was added to Cornelissen's (1996) graph of seed weight to initial seedling size, a very similar trend would be seen; that almost all seeds below 0.1 mg are gaining mass in the transition from seed to seedling, while almost all seeds above

100 mg are losing biomass. This agreement in results suggests a potential ubiquitous rule among trees, where larger seeded species produce larger seedlings, but smaller seeded-species lose less mass in relative terms during the transition from seed to seedling.

Typically researchers investigate mean sizes for seed and seedling per species. We followed the exact masses for each individual seedling, rather than using species means. Although this prevented us analyzing dry seed mass, it did enable separating species and size, highlighting that this relationship is largely driven by size rather than species differences. Despite the small-seeded species loosing less mass, there can be advantages of greater seedling size, such as the ability to intercept more light than those below you, or for deeper rooted seedlings the ability to adsorb more water in times of low soil water potential (Turner 2001).

Growth Performance along a light gradient

Most species increased their total final mass sharply between 0.3 % light and 3% light, and began to plateau towards 18% light (figure 4 & 5). Generally the relationship between final mass in the mid light and high light was positive; if a species grew well in high light, it also grew well in the mid light (figure 10). This positive relationship between growth in the high and mid light treatments was observed regardless of the metric employed; mass, AGR or RGR (Figure 10). A perfect negative relationship would indicate a complete reverse in rank for all of the species. However this is not to say that a positive relationship indicates that there are not substantial rank changes, as can be see from figures 9 and 10. Sack and Grubb (2001) considered seven studies investigating the growth of woody seedlings at high and low irradiance by calculating the RGR using the final harvest. Four of the studies showed a positive correlation between growth at low and high irradiance, two showed

no pattern and only one study showed a negative trend. However the study showing a negative trend investigated the growth of both pioneer and shade-tolerant species (Agyeman et al. 1999). The species in our study are all shade-tolerant climax species and are much more ecologically similar than those in the study of Agyeman et al. (1999). We would therefore not expect such a dramatic shift in relative species performance as with their study. A more recent study by Bloor and Grubb (2003) focussing on the growth of 15 shade-tolerant tropical trees, also showed a strong positive correlation between growth in high and low light, suggesting that this is more likely to be the trend we would expect from our study investigating shade-tolerant species.

While the positive relationship between growth in the high and mid light treatments (figure 10) demonstrates that there is not a complete rank reversal of the species we studied, there are still individual cross-overs, or changes in rank between some of the species (figures 5,7,9, tables 1,2,3).

Perhaps all that is required for coexistence of many tree species in a tropical rain forest is some cross-overs in rank, rather than a complete negative correlation between growth in high and low light environments. Bloor and Grubb (2003) also found a number of changes in rank order despite a strong positive relationship between growth in low and high light conditions, however they did not have any species that had a poorer performance in their lower light treatment. In our study, a small number of species had lower growth rates in the high light treatment. In terms of total final mass and AGR only two species, *Shorea leprosula* and *Shorea macroptera,* performed less well in the high light treatments, yet this drove substantial rank changes in total final mass and AGR. In relative growth *Shorea johorensis* and *Shorea macroptera* were both growing faster in the mid light

treatment, while *Shorea leprosula* had the same rgr in both the mid and high light treatments – this resulted in a greater number of changes (than for total mass and AGR) in rank efficiency, but it would take time before these differences result in one species out-competing another.

Sack & Grubb (2001) believe that the differing results and strengths of relationships seen in the literature could be due to inconsistency in lengths of study's and harvest intervals. Our study only examines a snapshot of seedling establishment. It is possible that a study of a few years longer, following the growth period of seedlings into saplings would uncover a greater number of more pronounced cross-overs. However, it is logistically difficult to carry out nursery experiments that span longer periods of time. Ideally accurate nursery experiments with biomass should be backed up with longer term field studies. However we can use the growth estimates from our experiments to project into the future.

If the average RGR that was observed during the course of the experiment was maintained then it would take almost one and a half years (514 days) for the smallest and fasting growing species – *Hopea nervosa* – to overtake the largest and one of the slowest growing species – *Shorea macrophylla*. This is a relatively short time in terms of the regeneration of forest seedlings.

There is a strong debate in the literature over the role of light in the coexistence of diversity in forest communities (Sack and Grubb 2001, Kitajima and Bolker 2003, Sack and Grubb 2003). On the one hand – as we have examined here – species specialize their growth to different light environments; while on the other hand light may play a role in the coexistence of diversity through a trade-off of growth in the light versus survival in the shade. There is mounting support, evident in the work of Kitajima and colleagues, that a growth and survival trade-off is of more likely than

growth under different light conditions (Kitajima 1994, Kitajima and Bolker 2003). In our study almost all of our seedlings survived, so it is not possible to directly test this hypothesis with this dataset. However it should be noted that these two hypothesis are not necessarily mutually exclusive and they could well be acting in unison.

Seed size mass and growth rate relationships after one year of growth

After one year of growth the largest seeded-species still had the greatest biomass in all three light treatments, even after accounting for their larger initial size, the larger individuals had grown more in all light treatments. However per gram of existing biomass the larger seeded-species are growing slightly slower than the smaller-seeded species. In relative terms the smaller-seeded species are much more efficient than the larger-seeded species.

In a recent meta-analysis Poorter and Rose (2005) found that seed size affects the growth of seedlings through a negative relationship between seed size and growth rate and concluded that seed size is a good surrogate for the shade-tolerance of rain-forest tree species. While (Poorter and Rose 2005) found that a relationship between seed-size and growth rate has been shown in many studies at the seedling stage, there is less evidence that this relationship continues throughout the life history of tropical trees.

During the course of this experiment the size advantage bestowed from a greater maternal investment in seed size enables the larger seeded individuals to grow faster – in absolute terms - and stay larger than the smaller-seeded species. However, the greater efficiency implied by the RGR results would suggest, that

ultimately the smaller seeded individuals may be able to catch up and outgrow the larger seeded-species.

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Chapter 2

Adjusting for differences in initial size in the analysis of growth

Abstract

Relative growth rate declines with plant size and is therefore size dependant. To combat this, some researchers add initial seedling size into the statistical model before the term for species identity. Thus species effects can be compared after initial seedling size has been taken into account. The RGR of each individual can then be assessed at a common size. This enables the RGR's of species of differing sizes to be compared across a common size. Using the same dataset as in the previous chapter, we analysed each of the size corrected metrics of growth (final size, AGR, RGR).

As expected, RGR was negatively correlated with initial size while both AGR and total mass were positively correlated with initial size. Size correcting RGR and AGR caused minimal differences to the species cross-overs in growth rate in response to the light gradient. The relationship between size corrected RGR and AGR in the mid and high light treatments showed a similar positive relationship as it did without size correction. In order to highlight how size effects can dominate mean species RGR's; we size corrected RGR to the smallest seedling size, the mean size, and the largest seedling size. Taking account of initial size differences completely removed any negative seed size - RGR relationship, regardless of which common size the RGR was corrected to. The AGR seed size relationship was dependant on the light treatment, being negative in the low light treatment, and positive in the mid and high light treatments.

All species had a higher RGR when corrected to the smallest seedling size, than when corrected to the maximum seedling size – reflecting a general slowing of relative growth with increased size. Shifts in growth rate when corrected at the mean size were species dependant. This emphasizes how the slower growth rates of larger

individuals and the faster growth rates of smaller individuals are largely driven by size as opposed to species identity. This suggests that the commonly seen negative seed size - RGR relationship is a product of differences in initial seedling size, rather than physiological species differences. However, there are additional species-specific effects.

The relationship between total mass and initial size was more complex than for the other two metrics as it differed for each species and light treatment combination. The size correction of final mass resulted in the unlikely prediction of negative masses for some species. The result of the negative mass prediction being a much greater change in the number and nature of species cross-overs for final mass in response to the light gradient. The relationship between mass and seed size was dependant on the light treatment; there was no relationship in the low and mid light treatments, and a weakly positive relationship in the high light treatment.

Differences in size between the smallest and largest seedlings were substantial. These differences often caused the predicted values from the initial size relationships to be far beyond the regression. This disparity represents a large methodological draw-back, especially when differences in mean species size are large – as it may result in inaccurate predictions.

In some species - treatment combinations there was a negative relationship between final mass and initial size. Each species treatment combination was only replicated with 5 individuals; hence these relationships may be driven by noise in the data as opposed to a true negative relationship. However, the separate initial size - total mass relationships for each species-treatment combination are still used in the size

correction, and may contribute to the inaccurate predictions. Both of these issues emphasize how the method of using covariates for size-correction is a 'get-around' for dealing with the difficulty of size dependant growth rate measures. The problem is not addressed directly, hence in some situations the metric operates well, while in others in can create spurious or incorrect results.

Introduction

Diminishing relative growth rate with increased size is a widespread pattern amongst vascular plants (Metcalf et al. 2006). Counter to what is sometimes thought, RGR measures are therefore size-dependant. Different species often vary in their mean size - especially at the early seedling stage when initial differences in seed size are still apparent. This means that when comparing species of different sizes the size effect is confounded with other potential differences in growth and physiology. In order to make fair comparisons between species that are not confounded with size it is important to account for size differences before species are compared (MacFarlane and Kobe 2006).

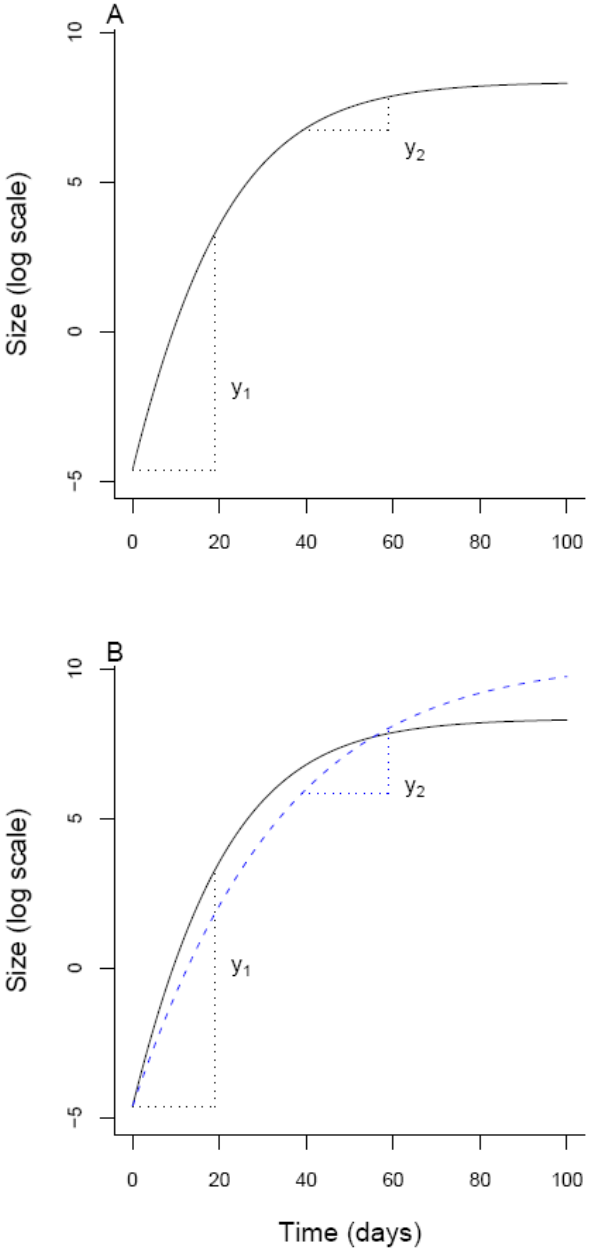
If plant growth was linear, then absolute growth rate would be constant, and as a measure would allow a fair comparison of different sized individuals. For example if a 1 gram plant grew to 2 grams in the course of a week, it would have an absolute growth rate (AGR) of 1 gram per week. If we expected the plant to grow at the same constant linear rate, then when it is 10 grams in size, it would grow to 11 grams in the course of a week, and therefore still have an absolute growth rate of 1 gram per week. So if plants grow at a constant rate then it would be fair to compare a 1 gram and 10 gram plant using AGR.

However, as we have seen in the previous chapter, AGR only accounts for size differences by subtracting the starting point, but does not account for larger plants ability for a greater increase in size. We would expect plants to invest some of this new mass into photosynthetic material, i.e leaves, and would therefore expect the larger plant to have a greater capacity to increase its biomass.

AGR is useful for comparing absolute difference, but it is in some sense unfair – one plant doubled it's biomass while the other only increased by 10%. Relative Growth Rate (RGR), by comparison examines growth on a percentage basis. Here the 1 gram plant is growing at a RGR of 0.69 grams per gram per week, whereas the 10 gram plant is only increasing its mass by 0.1 gram per gram per week. In one way this metric is fairer, as it takes into account the greater per gram growth efficiency of the smaller plant. However, now we are essentially assuming that the plants are growing at a constant exponential rate. Exponential growth occurs when an organism is growing at a constant multiple of its starting mass. The problem with this metric is that not all of the biomass is productive. This is especially the case with a tree, when a large proportion of the mass is dead wood.

Hunt (1990) presented growth data from a wide variety of organisms, showing that bigger and more complex organisms have lower RGR's. Hunt (1990) suggests RGR decreases with size as morphological adaptations are required to sustain larger systems. It is easy to imagine a situation where a simple organism, such as an alga, could grow exponentially. However an organism, such as a tree, which needs to allocate a large amount of its carbon to structural tissue, is less likely to be able to grow at an exponential rate. As plants grow the proportion of structural tissue increases, this results in an ontogenic decline in RGR (Hunt 1982), we therefore can't expect RGR to describe more than initial plant growth. Recently Turnbull et al. (2008) also highlighted that a higher RGR will be observed for smaller individuals even if they are the same species and have identical growth periods (Figure A). They showed how this could result in a spurious relationship between RGR and seed size. Here we investigate how simple methods of correcting for size differences affect the relationship between seed size and growth rate measures.

Metcalf et al. (2006), calculated their measure of RGR for the smallest and largest sizes common to all species. Unfortunately this was not possible for our dataset as there is no overlap between the smallest and largest species. We corrected RGR to the smallest, largest and mean sized seedling from the entire dataset. We are cautious in our interpretation as the resulting predictions are far away from the range of data for many of the species.


A
B
Size (log scale)
Time (days)
y1
y2
10
5
0
−5
0
20
40
60
80
100

◄ Figure A: Conventional RGR can be insensitive to growth rate differences when sizes are different and log size declines with time. (redrawn from Turnbull et al.)

Figure legend as per Turbull et al. 2008.
If instantaneous RGR (relative growth rate) declines with size, species that begin growth at a smaller mass will always have higher average RGR (y1 . y2) whether (A) two species have exactly the same instantaneous growth rate at a given size (in this case a Gompertz function) or (B) small-seeded species actually grow faster for a given size and can therefore outgrow the larger-seeded species at least initially. A negative correlation between RGR and initial size cannot therefore distinguish between these two alternatives.

Methods

The dataset is identical to the one described in chapter 1, therefore the methods for chapter 1 describe how it was collected.

Statistical analysis

In this chapter we analyze the dataset using linear mixed effect models, and attempt to correct for size differences for individuals before comparing treatment and species effects.

We fit a model such as RGR ~ initial size * Species * Treatment. Initial size was always kept as the first term in the model. Stepwise backward deletion using AIC as the criteria was used to determine the best model. Often interactions between initial size and other terms where not required. As the variance varied significantly with species and RGR, we used the varFunc argument in lme to account for differing variances.

The second stage was to predict new values of the response variable using the model and fixing the initial size to a common size; such as the mean. Mean's of the new predicted data were then calculated for each species treatment combination and explored in response to seed size graphically and using basic linear models.

Results

Guide to results section

For each metric (total size, AGR, RGR) we present the relationship between initial size and the metric. We then present the graphs of that size corrected metric in response to light for each species; the relationship between the size corrected growth rates in the high and mid light and finally the relationship between the mean species metrics in response to seed size. We start with RGR and then AGR as the relationship with initial size is more simple for these metrics than it is for mass.

Results

Relative growth rate is strongly negatively affected by initial mass (Figure 1, $F_{1, 138}$ = 51.34, P<.0001). Adding in the initial size term has greatly increased the significance of light treatment ($F_{2, 8}$ = 152.05, P<.0001), and decreased the effect of Species identity ($F_{11, 138}$ = 5.21, P<.0001) compared to the analysis in chapter one with no term for initial size (page 20).

RGR's where then recalculated from this model for each individual using the mean initial size. Means for each Species treatment combination were recalculated and are presented in Figure 2. Although taking account of initial size before comparing species and treatment differences makes a substantial difference to the significance the general pattern of between species differences in RGR's compared at a common mean size is very similar (Figure 2, Table 1) to before size correction.

There was a strong effect of initial size, which greatly reduced the effect of species differences. In other words much of the difference bwtween species is due to the

general slowing of growth with increasing size. In figure 3, we present the mean RGR for each species comparing the growth in mid light against growth in the high light treatment. We have corrected the RGR's to the minimum size, mean size, and maximum size in order to highlight how the effect of size changes species means. On the far left panel of Figure 3, each species mean RGR – in blue - has been calculated from individual RGR's corrected to the size of the smallest individual. The grey points show the uncorrected RGR's. When RGR's are corrected to the smallest size - the species means for all species in both light treatments have increased. This highlights how the smaller species are growing faster as a result of their size, rather than their species identity. The shifts when correcting RGR's to the size of the largest individual are substantial and greatly reduce the RGR of all species in both light treatments (Figure 3, far right panel). This highlights how the lower RGR of the larger species is explained more by differences in size than by species identity. The relationship between growth in the mid and high light remains unchanged regardless of which common size is used for correction.

Absolute Growth Rate increases with greater initial mass (Figure 4, $F_{1, 138} = 124.34$, $P<.0001$). Again, as in the case of the size corrected RGR's, the cross-overs and changes in rank, do not change substantially compared to prior to the size correction. However *Shorea macrophylla* does worst in the low light treatment, compared to best before the adjustment for size and *Hopea nervosa* has a considerably lower AGR in the mid and high light treatment compared to before the size adjustments. In other words, the high absolute growth rates of these species in these light treatments is mainly a function of their large initial size.

Total final mass is strongly positively affected by initial mass (Figure 6, $F_{1, 103}$ = 1308.53, P<.0001), but this relationship is different in each of the light treatments (Figure 7, $F_{2, 103}$ = 86.19, P<.0001). The relationship between total final mass, and initial mass is also different for species (Figure 8, $F_{11, 103}$ = 3.61, P<.004), but is also different for some species light treatment combinations (Figure 8, $F_{22, 103}$ = 5.37, P<.0001). It appears from Figure 8 that some of the separate species treatment slopes are not well supported by the data, however, removing the three way interaction caused a difference in the AIC of over a 100 points, therefore we retained the 3-way interaction. This results in the slopes for size correction for mass being different for each species light treatment combination.

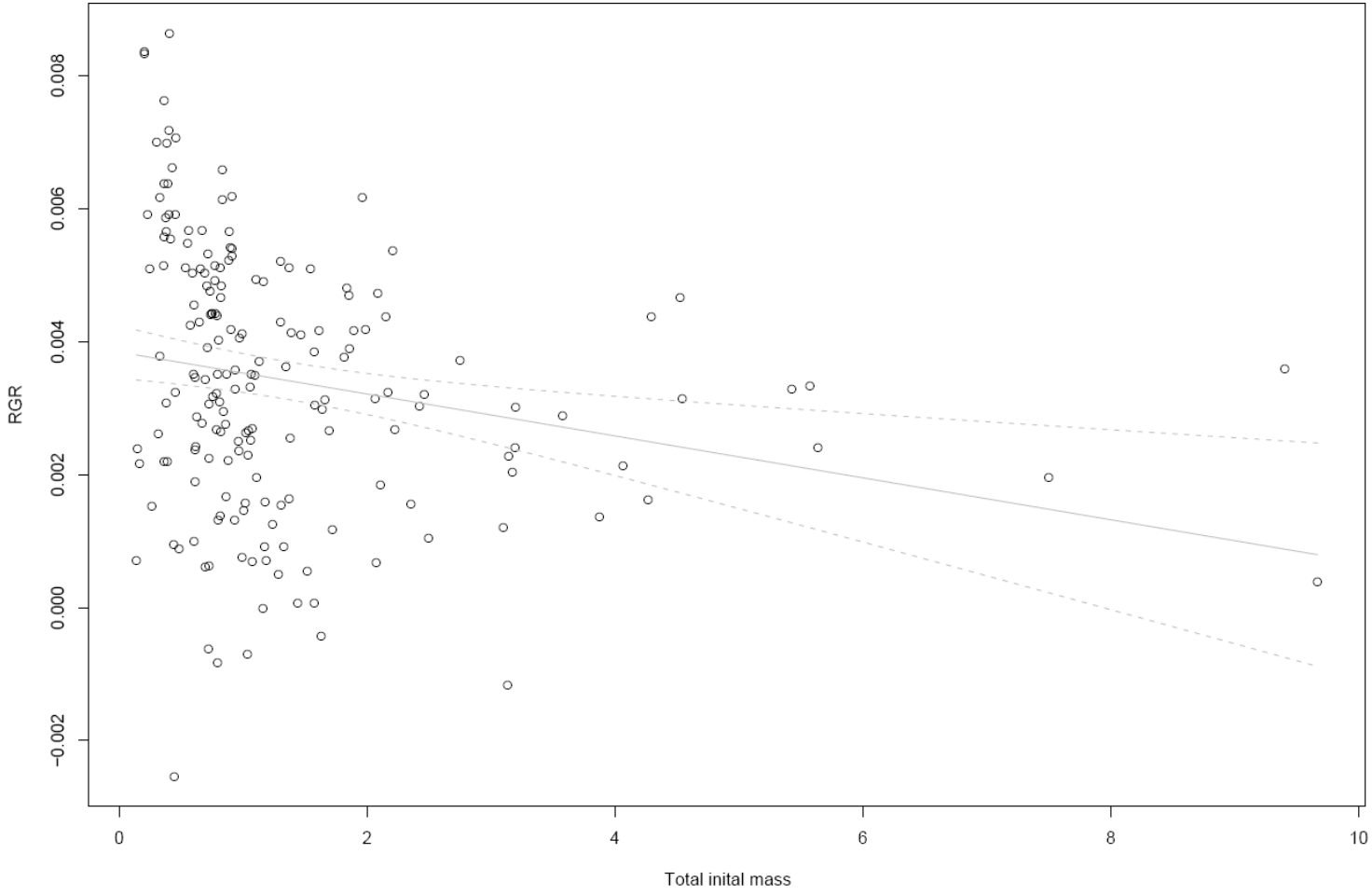
The size corrections resulting from these mass, species and light treatment interactions result in some species changing rank considerably in final mass compared to before size correction (Figure 9). *Hopea nervosa*, which had the second lowest mass in the uncorrected analysis, now has the second highest mass in the high light treatment (figure 9). *Shorea macrophylla, Shorea macroptera* and *Hopea nervosa* have predicted negative mass's in the mid light treatment (figure 9) resulting in dramatic cross-overs (Table 3).

Figure 10 shows how the size correction has affected the relationship between growth in the high light and growth in the mid light for each of the three metrics. The species with higher AGR's now have lower AGR estimates and compared too the previous positive relationship (R^2=0.73, $F_{1, 10}$ = 27.0, P>0.001). The estimates of total final mass for a few species have shifted to high mass in the high light and near zero mass in the mid light – this completely removes the positive relationship (Figure 10 third panel, R^2= 0.083, $F_{1, 9}$ = 0.91, P=.363).

Here we have size corrected most of our analysis to the mean starting size. This is problematic as the smallest individuals don't reach this size by the end of the experiment, and the largest individuals start much bigger than the mean starting size. There is no real solution to this problem, but it could be prudent to also size correct to the smallest and largest size seedling in order to understand a little about how predicting far to one side of the regression. Figure 11 shows RGR size corrected to the minimum, mean and maximum starting mass. The relationship between RGR and seed size is non existent regardless of which size the individual RGRs are corrected too, however the scale is quite different.

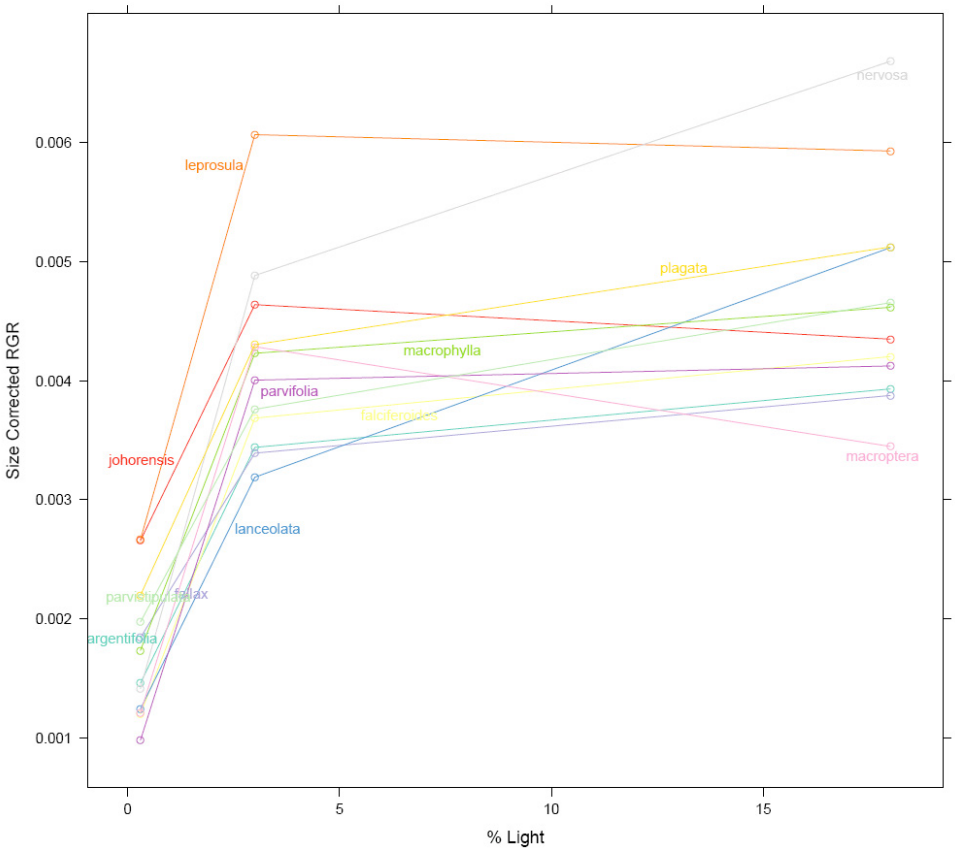
Even after correcting for size difference there is still a positive relationship between AGR and seed size in the mid and high light treatments but a negative relationship in the low light treatment (Figure 12, $F_{2, 30} = 11.93$, $P<.001$).

There is no relationship between estimated mass for seedlings that have been predicted to start at a mean size in the low and mid light treatment, but there is a positive estimated mass seed size relationship in the high light treatment (Figure 12, $F_{2, 30} = 4.30$, $P<.05$)


RGR
0.008
0.006
0.004
0.002
0.000
−0.002
0
2
4
6
8
10
Total inital mass

◄Figure 1: Relative Growth Rate declines with initial mass

Relative Growth Rate in grams per gram per day for each individual seedling against the total initial mass of each individual.



RGR Rank Order

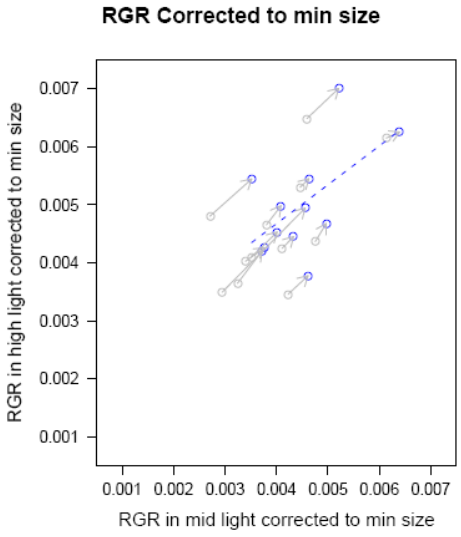
	Light Treatment		
Species:	Low	Mid	High
Shorea leprosula:	1	1	2
Shorea johorensis:	2	3	7
Hopea plagata:	3	4	3
Shorea parvistipulata:	4	8	5
Shorea fallax:	5	11	11
Shorea macrophylla:	6	6	6
Shorea argentifolia:	7	10	10
Hopea nervosa:	8	2	1
Dryobalanops lanceolata:	9	12	4
Shorea macroptera:	10	5	12
Shorea falciferoides:	11	9	8
Shorea parvifolia:	12	7	9

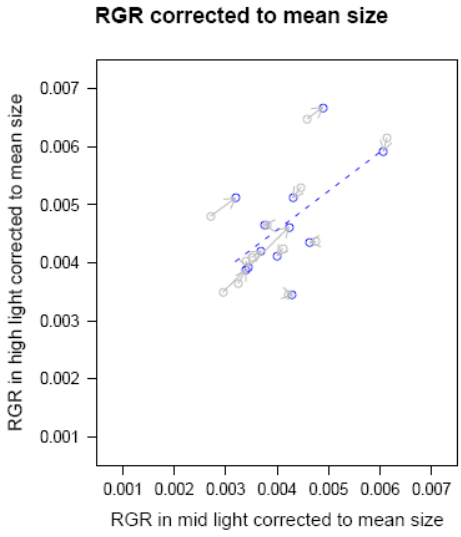
◄Figure 2: Cross-overs in size corrected RGR

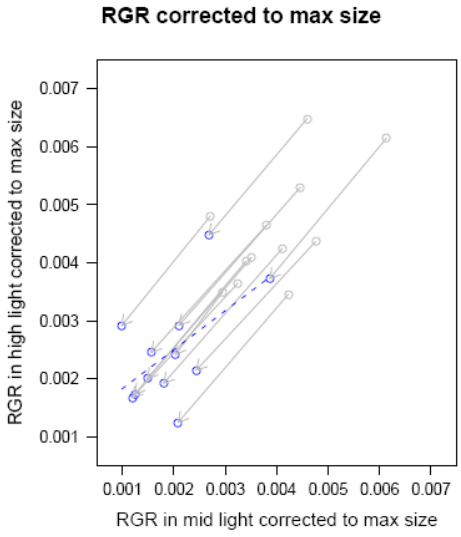
The mean RGR's for each species light treatment combination size corrected to a common mean size. Means for each light treatment are joined with a straight line to give an idea of where a cross-over might occur.

◄Table 1 Changes in rank order in response to light treatment

Species names are ordered according to their rank in the low light treatment. The numbers indicate how each species changes rank as the light treatment changes

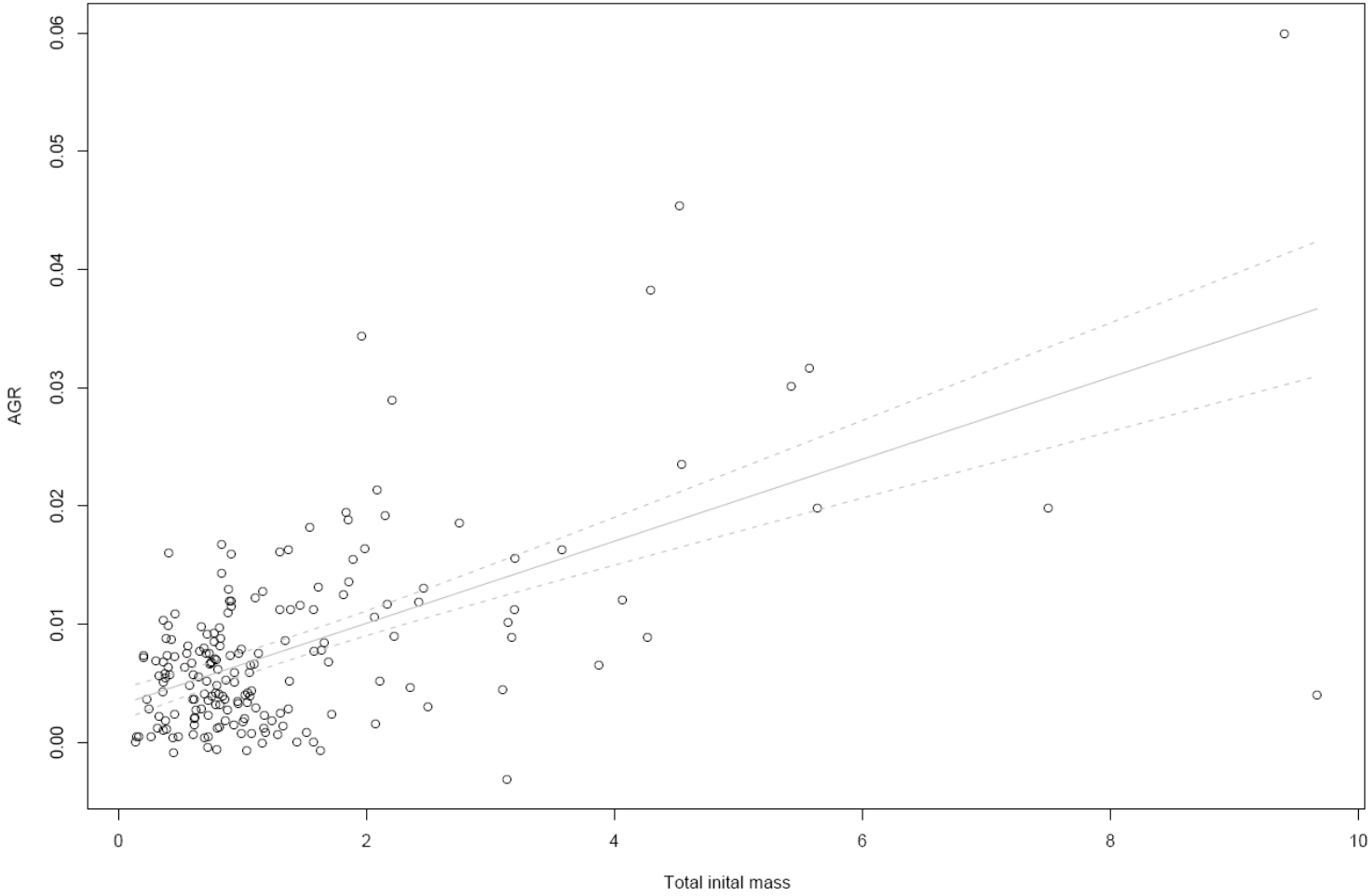

RGR Corrected to min size
RGR in high light corrected to min size
RGR in mid light corrected to min size
0.001
0.002
0.003
0.004
0.005
0.006
0.007


RGR corrected to mean size
RGR in high light corrected to mean size
RGR in mid light corrected to mean size
0.001
0.002
0.003
0.004
0.005
0.006
0.007


RGR corrected to max size
RGR in high light corrected to max size
RGR in mid light corrected to max size
0.001
0.002
0.003
0.004
0.005
0.006
0.007

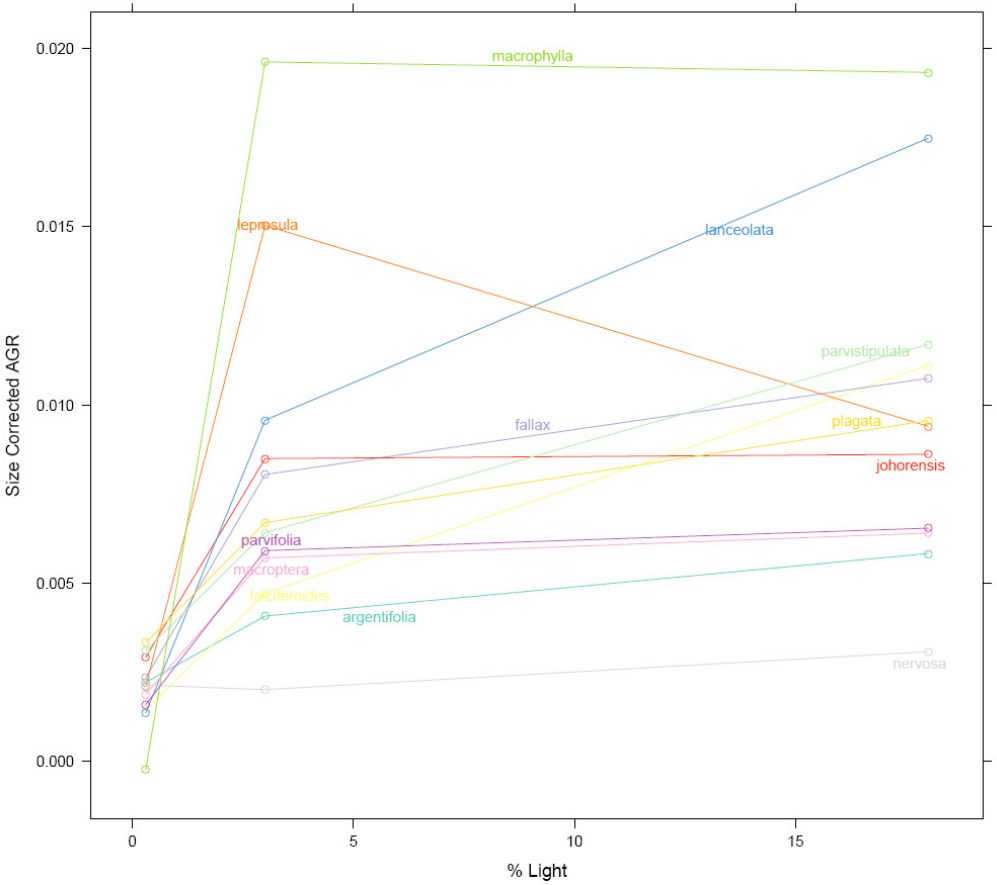
◄ Figure 3: The relationship between RGR in the high and mid light corrected to either the smallest individual in the dataset; the mean initial size; or the largest individual in the dataset.

Blue points represent the means of size corrected RGR's. Grey points represent the raw RGR values, grey arrows show how far the RGR has shifted after size correction and indicate where the previous RGR was for that species. The blue dotted line represents the regression for the size-corrected points


AGR
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0
2
4
6
8
10
Total inital mass

◄ Figure 4: The relationship between AGR and total initial mass

Regardless of species or light treatment, AGR is strongly positively affected by the total initial mass of the individual.



AGR Rank Order

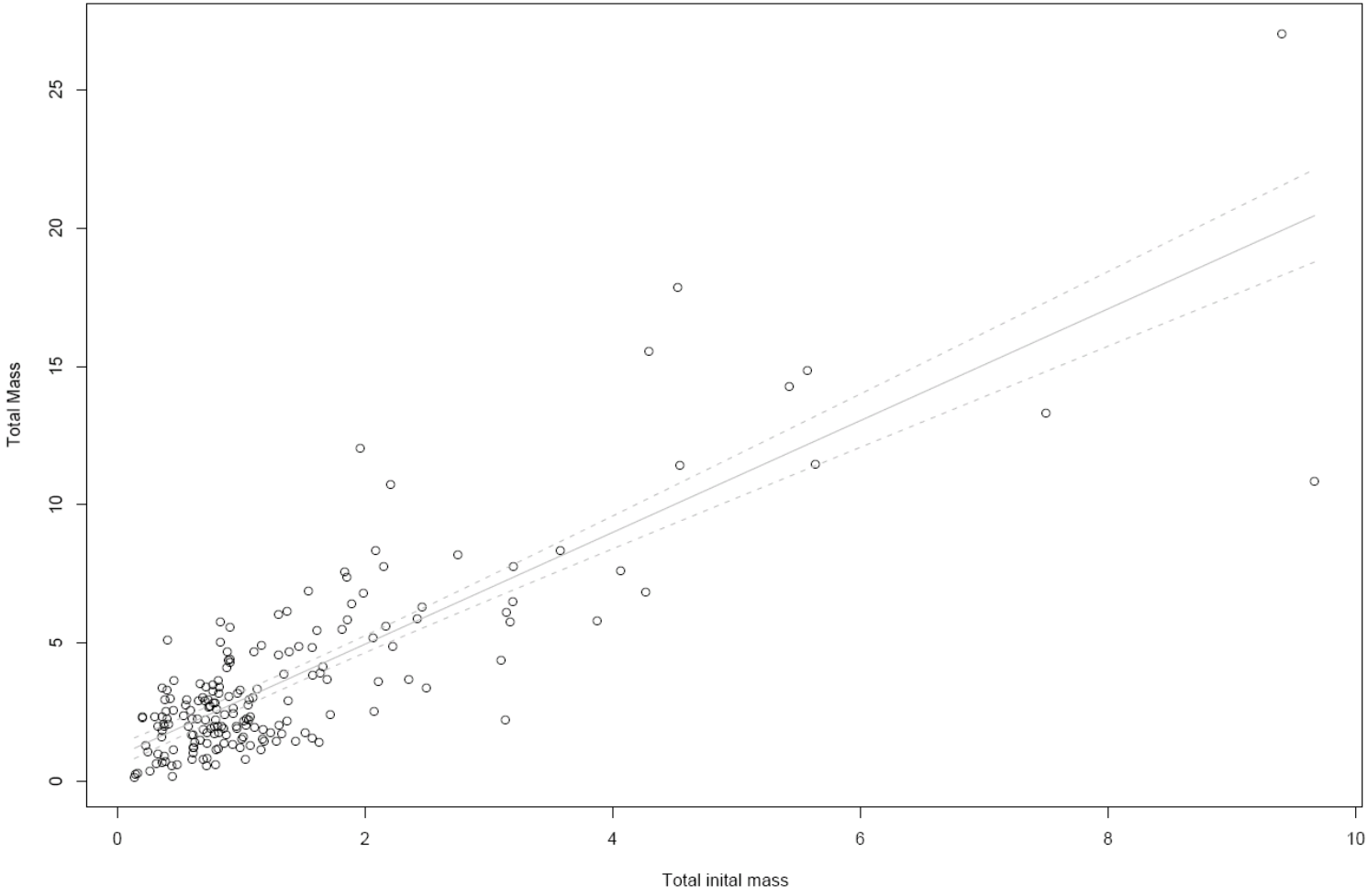
	Light Treatment		
Species:	Low	Mid	High
Hopea plagata:	1	6	6
Shorea parvistipulata:	2	7	3
Shorea johorensis:	3	4	8
Shorea fallax:	4	5	5
Shorea argentifolia:	5	11	11
Hopea nervosa:	6	12	12
Shorea leprosula:	7	2	7
Shorea macroptera:	8	9	10
Shorea parvifolia:	9	8	9
Shorea falciferoides:	10	10	4
Dryobalanops lanceolata:	11	3	2
Shorea macrophylla:	12	1	1

◄ Figure 5: Cross-overs in size corrected AGR

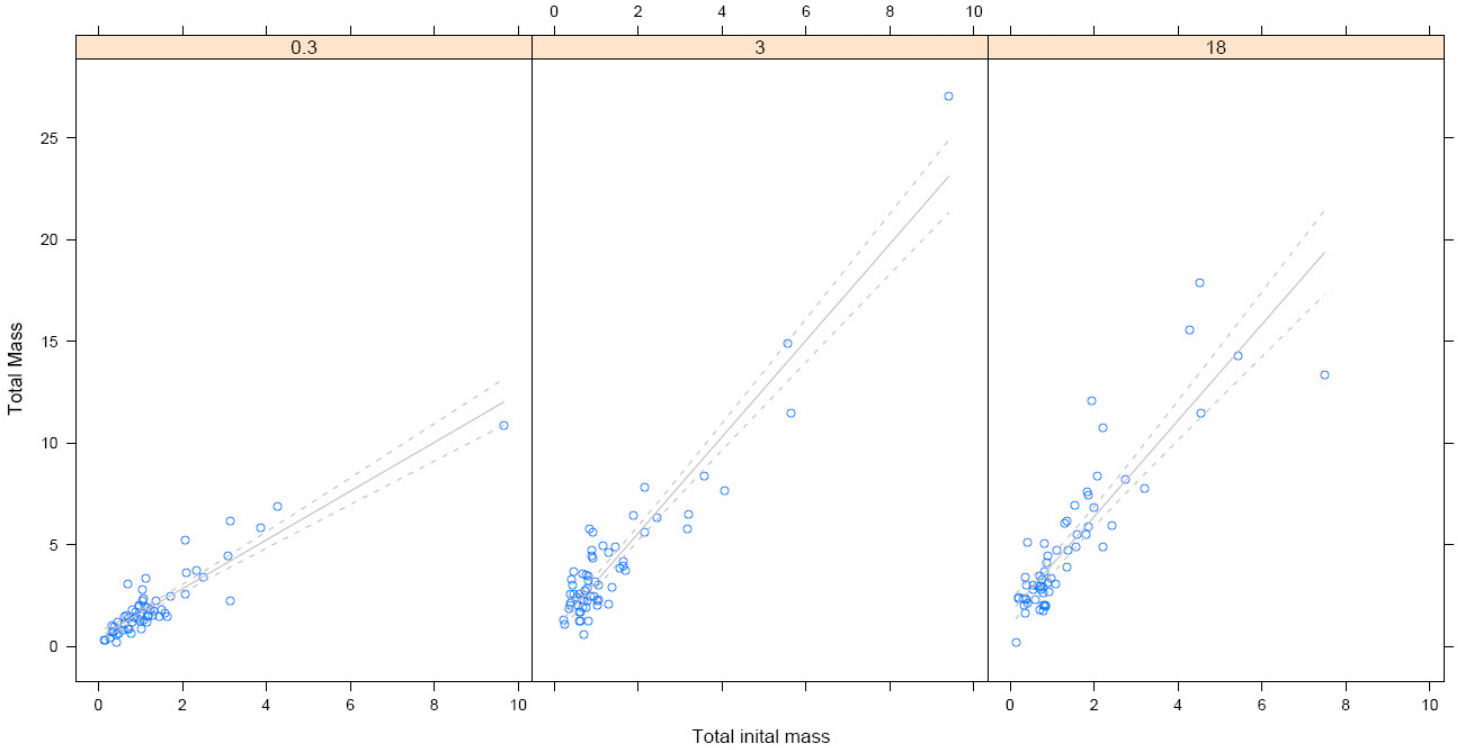
The mean AGR for each species after size correction for each of the three light treatments. Means for each light treatment are joined with a straight line to give an idea of where a cross-over might occur.

◄ Table 2: Changes in AGR rank order in response to light treatment

Species are ordered according to their rank in the low light treatment. The numbers indicate how each species changes rank as the light treatment changes

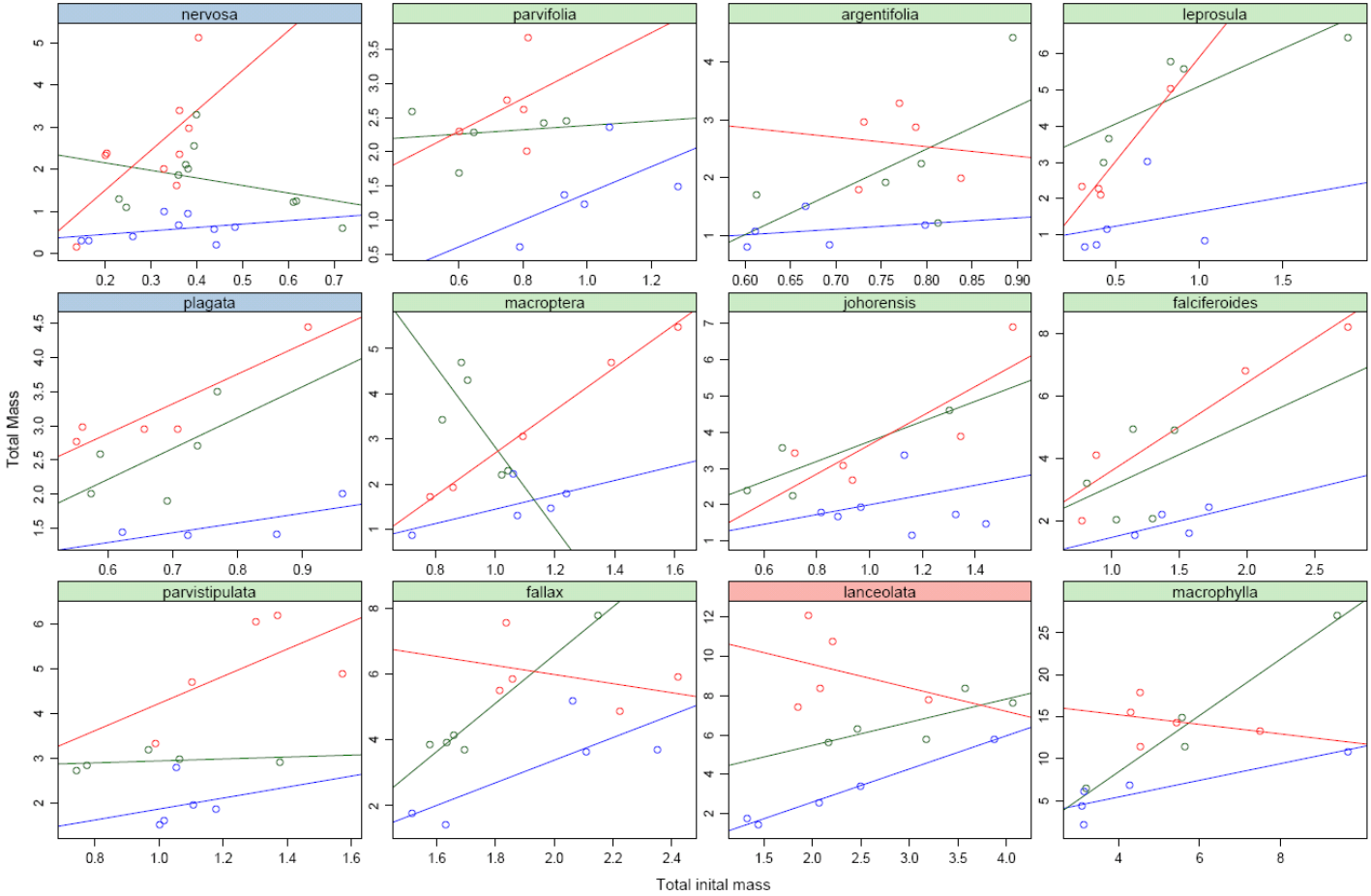

Total Mass
0
5
10
15
20
25
0
2
4
6
8
10
Total inital mass

◄ Figure 6: Total final mass is strongly positively affected by initial mass


0.3
3
18
Total Mass
Total inital mass
0
5
10
15
20
25
0
2
4
6
8
10

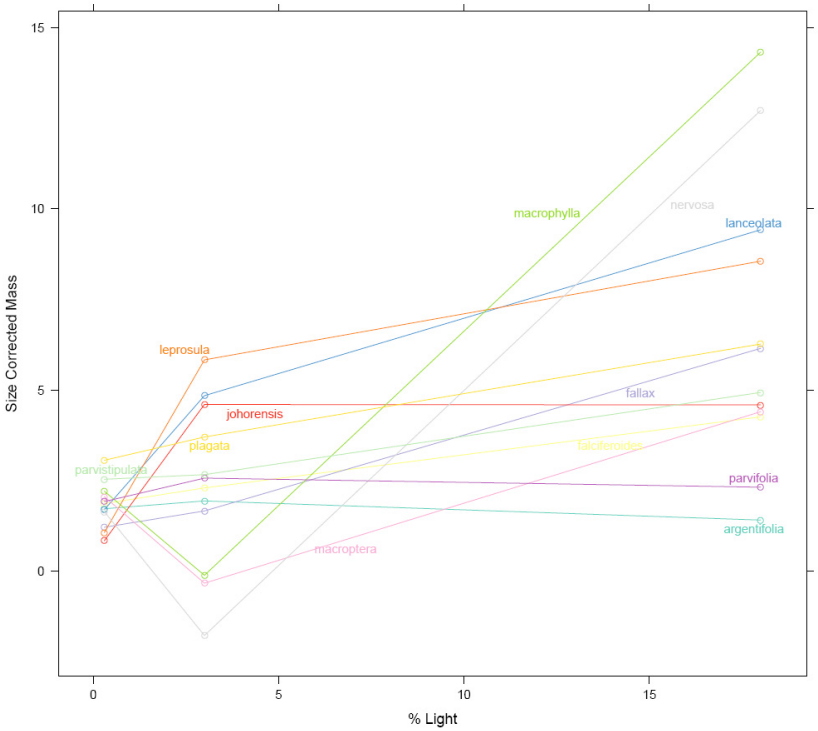
◄ Figure 7: The affect of initial mass on Total final mass is strongly affected by light treatment

There is little or no growth for the largest individual in the lowest light treatment.


nervosa
parvifolia
argentifolia
leprosula
plagata
macroptera
johorensis
falciferoides
parvistipulata
fallax
lanceolata
macrophylla
Total Mass
Total inital mass

◄ Figure 8: The relationship between initial mass and Total final mass is different for each of the Species and light treatment combinations.

Red represents the high light treatment; green the mid light treatment and blue the low light treatment.



Mass Rank Order

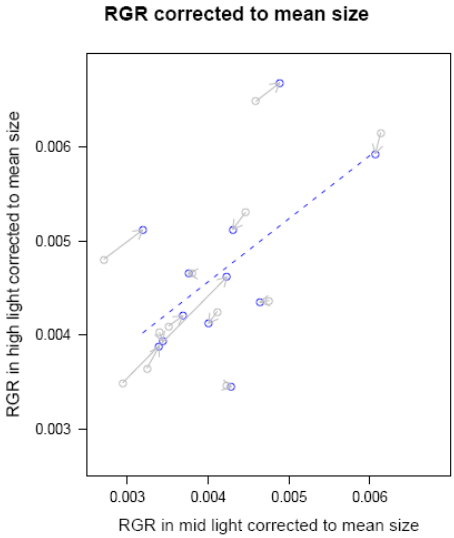
	Light Treatment		
Species:	Low	Mid	High
Hopea plagata:	1	4	5
Shorea parvistipulata:	2	5	7
Shorea macrophylla:	3	10	1
Shorea macroptera:	4	11	9
Shorea parvifolia:	5	6	11
Shorea falciferoides:	6	7	10
Shorea argentifolia:	7	8	12
Dryobalanops lanceolata:	8	2	3
Hopea nervosa:	9	12	2
Shorea fallax:	10	9	6
Shorea leprosula:	1	11	4
Shorea johorensis:	12	3	8

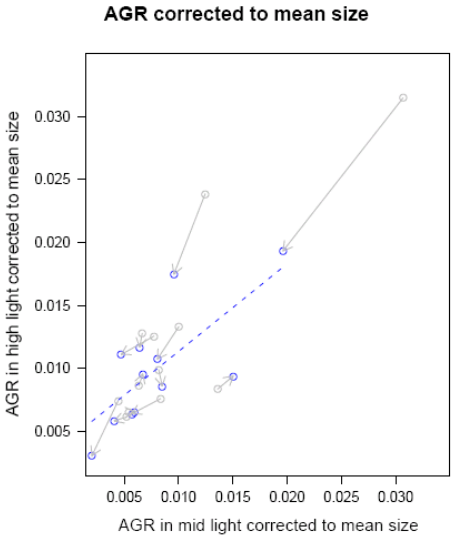
◄ Figure 9: Cross-overs in size corrected final mass

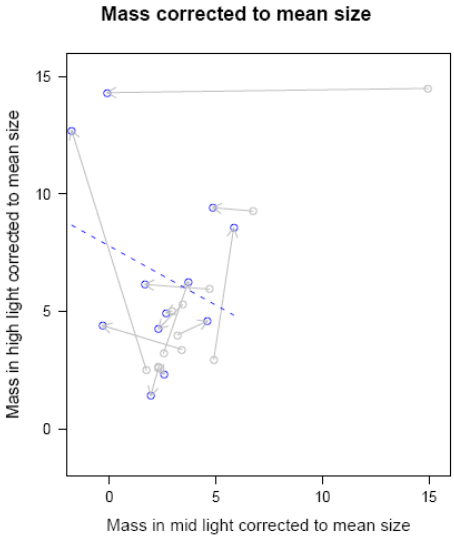
The mean mass for each species after size correction for each of the three light treatments. Means for each light treatment are joined with a straight line to give an idea of where a cross-over might occur.

◄ Table 3: Changes in AGR rank order in response to light treatment

Species are ordered according to their rank in the low light treatment. The numbers indicate how each species changes rank as the light treatment changes

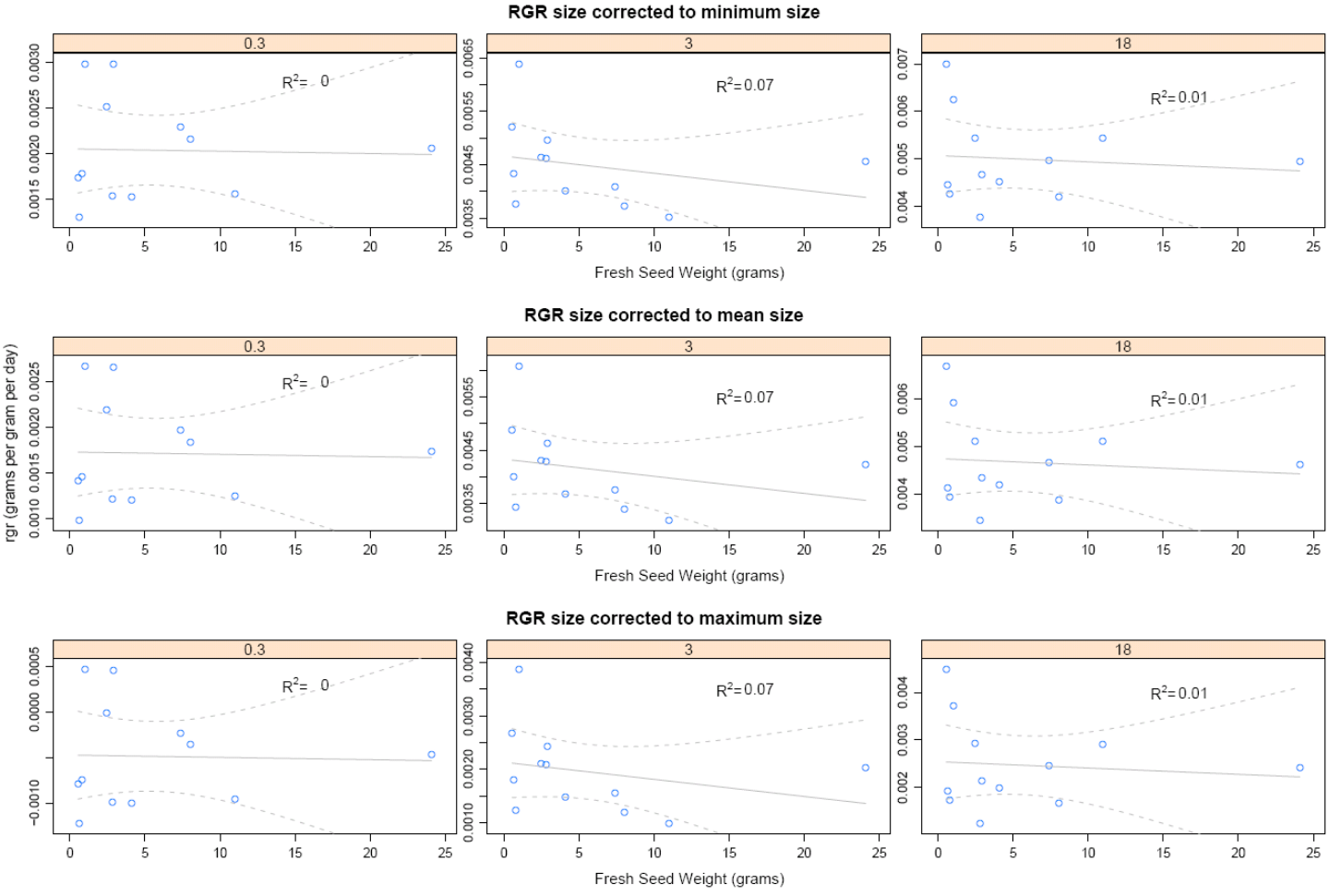

RGR corrected to mean size
RGR in high light corrected to mean size
RGR in mid light corrected to mean size
0.003
0.004
0.005
0.006


AGR corrected to mean size
AGR in high light corrected to mean size
AGR in mid light corrected to mean size
0.005
0.010
0.015
0.020
0.025
0.030


Mass corrected to mean size
Mass in high light corrected to mean size
Mass in mid light corrected to mean size
0
5
10
15

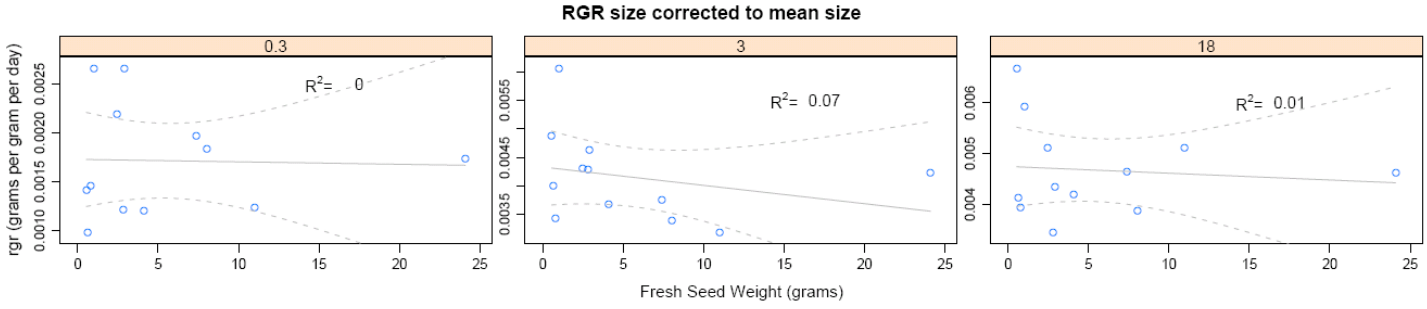
◄ Figure 10: The relationship between RGR, AGR and Mass in the high and mid light corrected to the mean of initial seedling mass.

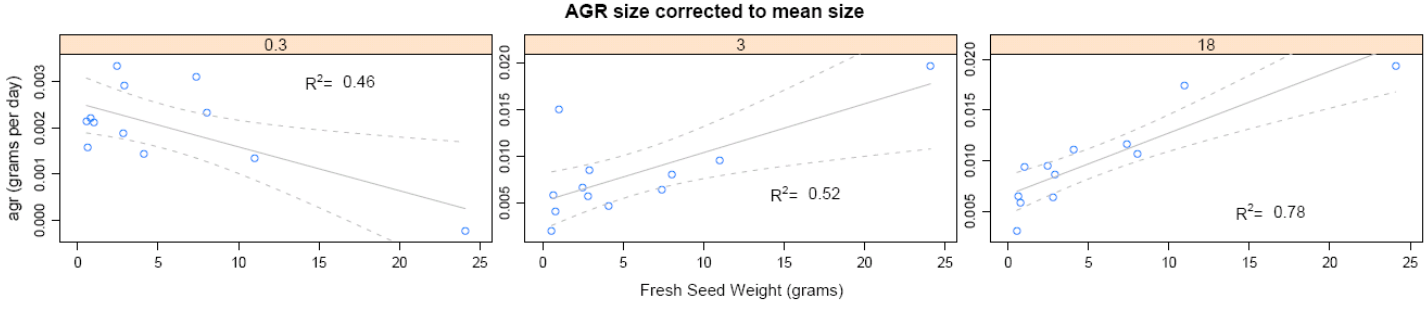
Blue points represent the means of size corrected RGR's for each species. Grey points represent the means of raw RGR values, per speceis, grey arrows show how far the RGR has shifted after size correction and indicate where the previous RGR was for that species. The blue dotted line represents the regression for corrected points.

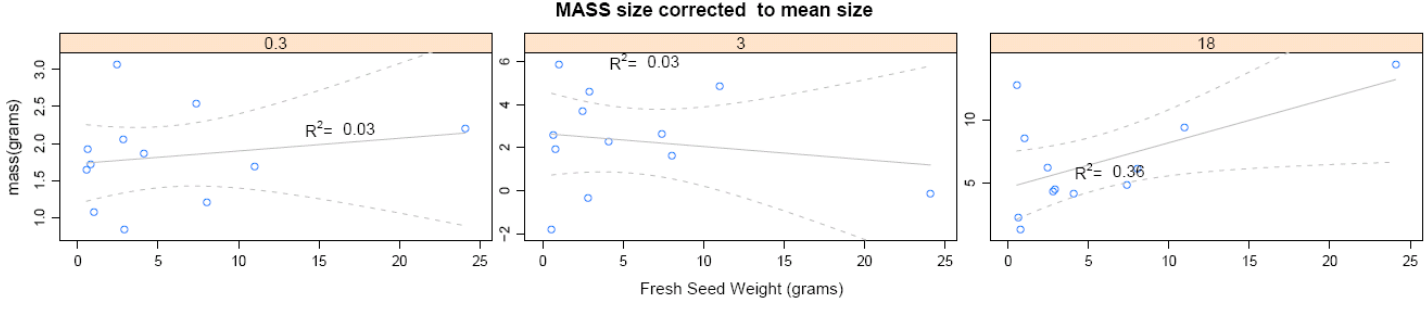

RGR size corrected to minimum size
0.3
3
18
R²= 0
R²=0.07
R²=0.01
Fresh Seed Weight (grams)
RGR size corrected to mean size
rgr (grams per gram per day)
0.3
3
18
R²= 0
R²=0.07
R²=0.01
Fresh Seed Weight (grams)
RGR size corrected to maximum size
0.3
3
18
R²= 0
R²=0.07
R²=0.01
Fresh Seed Weight (grams)

◄ Figure 11: Correcting RGR to a common size removes the seed size RGR relationship, regardless of which size RGR is corrected to.

The panels from left to right represent the low, mid and high light treatment. The three rows of panels show the RGR′s that have been corrected to the smallest size, the mean size and the largest sized seedling.


RGR size corrected to mean size
0.3
3
18
rgr (grams per gram per day)
R²= 0
R²= 0.07
R²= 0.01
Fresh Seed Weight (grams)


AGR size corrected to mean size
0.3
3
18
agr (grams per day)
R²= 0.46
R²= 0.52
R²= 0.78
Fresh Seed Weight (grams)


MASS size corrected to mean size
0.3
3
18
mass(grams)
R²= 0.03
R²= 0.03
R²= 0.36
Fresh Seed Weight (grams)

◄ Figure 12: RGR, AGR and Mass corrected to a common mean size.

The panels from left to right represent the low, mid and high light treatment. The three rows of panels, from top to bottom, show the means species RGR′s, the mean species AGR and the mean species mass respectively – each of which that have been corrected to a mean common size

Discussion

As expected, average RGR declines with initial size (figure 1). Initial seedling size is strongly positively correlated with seed size (figure 1, Chapter 1). Here we show that after correcting for differences in initial size we found no relationship between seed size and RGR (figure 11), suggesting that the negative seed size RGR relationship that has often been regarded as a ubiquitous physiological trade-off (Maranon and Grubb (1993) may in fact be largely driven be a general decline in RGR with an increase in size.

Maranon and Grubb (1993), found that the decrease of RGR with seed size, could be explained by a significant decrease in Specific Leaf Area (SLA) as seed size increased – smaller seeded species had thicker leaves, while larger seeded species had thinner leaves. While we cannot in fact be sure of cause and effect, it seamed evident to Maranon and Grubb (1993) that the negative seed size RGR relationship was due the thinner leaves of small seeded seedlings, intercepting more light per unit mass, resulting in a greater efficiency of growth, i.e both could be independently correlated with size and not with each other.

RGR can be split into three separate components; Net Assimilation Rate (NAR); Leaf Mass Ratio (LMR) and Specific Leaf Area (SLA) (Wright and Westoby 1999). Many studies across a variety of plant growth forms and biomes have shown the role of SLA in determining potential RGR (Wright and Westoby 1999) and it is thought to represent an almost ubiquitous relationship in plants. There is much less evidence, however, for the role of LMR and NAR in explaining variation in RGR (Lambers and Poorter 1992). If RGR is a size dependant measure then it could be

possible that SLA is also a size dependant measure and this effect is also an artefact of size. Niinemets (2006) found that studies have reported contrasting results about the role of leaf thickness in shade-tolerance. In temperate tree data Niinemets (2006) analysed, the relationship between SLA and shade-tolerance strongly depended on whether seedlings or saplings and trees had been investigated, and he believes ontogeny is important for changes in SLA. This supports our idea that SLA could be size dependant.

So if the negative seed size RGR relationship is explained by differences in seedling size, one possibility is that SLA explaining the variation is also a size affect. An alternative explanation is that some of these traits could be linked through species sharing a common ancestor rather than adaptive evolution. Some of these relationships have been tested taking into account taxonomic relatedness for example using Felsenstein's (1985) phylogenetically independent contrasts (PIC's).

The idea that small seeded species are faster growing and are more frequently found establishing in gaps is relatively well ingrained in ecological theory (Swaine and Whitmore 1988). However taking account of taxonomic relatedness of species in analysis rather than treating all species as independent data points, regardless of how closely related has challenged some of these ideas. Kelly and Purvis (1993) reanalysed a dataset from Foster and Janson (1985) that had found a relationship between seed size and establishment conditions in tropical woody plants. Once Kelly and Purvis (1993) considered taxonomic relatedness in the analysis there was no evidence for a relationship between seed size and establishment conditions in naturally occurring tropical tree species. Grubb and Metcalfe (1996) also found that within genera there was no difference between seed mass for light-demanding and

shade-tolerant species, and within families shade-tolerant genera had slightly larger seed masses. This suggests that these traits are shared because species in the study have shared a common ancestor rather than adaptive evolution resulting from an ecological trade-off. It is also possible that SLA could be linked to seed size and RGR in this way.

Metcalf et al. (2006) calculated their measure of a size independent growth rate at the smallest and largest size common to all species. This was not possible using our dataset because there wasn't overlap between the smallest and largest species. It may have been more conservative to correct our measures of growth the largest value of the smallest species, and the smallest value of the largest species, but this would have resulted in two values to continually consider. The mean seedling size was similar to the value of the smallest sized *Shorea macrophylla* and largest *Hopea nervosa* and is therefore comparable. For RGR we also corrected to the smallest and largest individual to emphasize how much this can change the absolute values. However, in studies where it is possible to follow the method of Metcalf et al. (2006), this would seem most prudent. Correcting RGR to the smallest size in the dataset is ecologically unrealistic as none of the individuals were ever this small, however it emphasizes how the smallest species are growing faster largely as a result of their size rather than their species identity or intrinsic physiological differences.

When the RGR's were corrected to the size of the smallest seedling in the dataset, then all of the mean species RGR's increased substantially, and when all RGR's were corrected to the largest seedling in the dataset the mean species RGR's were substantially reduced (Figure 3). This highlights the extent to which initial size dominates the mean species RGR's. Larger seedlings have lower RGR's than smaller

seedlings, regardless of species identity, however, there are also residual differences in RGR between species.

Correcting RGR for initial size completely removes any relationship between seed size and RGR regardless of which common size was used in the size correction (Figure 11). Although it is problematic that we are predicting out of the range of the data, and thus don't have complete confidence in the predictions, it is reassuring that using the middle or each end of the regression always removes the relationship. This strongly suggests that this method does in fact work, and the physiological explanation provided by Maranon and Grubb (1993) is incorrect. Even if these relationships are not completely explained by size differences, and there are residual species differences, we certainly need to understand these relationships independent of size in order to further elucidate these functional trade-offs.

Size correcting RGR had little effect on RGR rank orders rate in response to the light gradient and didn't result in much change in the raw RGR cross-overs (figure 2, table 1). Correcting for size also has limited effect on rank cross-overs in AGR (figure 5, table 2). The negative seed size RGR relationship seams to actually be an result of initial size differences – taking account of size removes the effect, whereas taking account of the species rank cross-overs for either RGR or AGR makes minimal difference. This suggests that the cross-overs we have observed in their first chapter are affected to a much lesser extent by the size bias. It is therefore important to remember that while size effects can dominate some trade-offs it doesn't necessarily mean that all patterns observed using RGR are incorrect.

AGR increases with an increase in initial mass (figure 4). When AGR was corrected for initial size differences the AGR seed size relationship was dependant on the light treatment, being negative in the low light treatment, and positive in the mid and high light treatments. For all the species there is very little growth in the dark light treatment. It is highly likely that all the seedlings are very close to the light compensation point (Barker et al. 1997), it would also make sense that the bigger you are the greater your net respiration, and a greater amount of respiration when photosynthesis is low would resulting in a lower net gain, and thus lower growth rate's for larger seedlings. In the mid and high light treatments there was still a positive relationship between size corrected AGR and initial size. Even after correcting for differences in initial size the larger seeded species have a considerably higher growth rate in absolute terms. Does this explain a greater physiological ability for larger seeded species to grow fast in higher light, or could this still be an product of size as AGR assumes a linear growth rate.

When we correct for initial size differences, we remove the part of the AGR signal that is explained by initial size. If in fact the seedlings are growing as a function of their size, either exponentially or sub or super-exponentially then the initial size effect could be multiplied through time. MacFarlane and Kobe (2006) pointed out that, theoretically, size effects should be neither constant nor proportional to size. It is very difficult to establish the true measure of size effects with this type of analysis. Nevertheless, MacFarlane and Kobe (2006), found that a model of AGR taking account of initial size fitted their tree growth datasets best and gave the most accurate predictions. This would suggest that this method is working and the estimates from our model are approximately correct. Thus it seams likely that the larger seeded species do in fact grow faster in Absolute terms even after

accounting for initial size differences. Following work by MacFarlane and Kobe (2006), other researchers are beginning to take account of tree size in their analysis. For example, in an analysis of tree growth in boreal forests Filipescu and Comeau (2007) found that adding in initial size as a second explanatory variable increased the predictive ability of growth models, and made analysis more sensitive to differences in other covariates.

The relationship between total mass and initial size, in different light levels, was complex, involving two-way and three-way interactions. Greater initial mass results in greater final mass (figure 6) but this relationship is steeper in mid and high light than in low light (figure 7). The relationship between initial and final mass was also different for each of the species light treatment combinations (figure 9). The size correction of final mass resulted in the unlikely prediction of negative masses for some species in the mid light treatment. This is probably mainly due to these separate slopes for each species treatment combination that aren't always well supported. The result of the negative mass prediction, after size correction, is a much greater change in the number and nature of species cross-overs for final mass in response to the light gradient. The relationship between mass and seed size was dependant on the light treatment; there was no relationship in the low and mid light treatments, and a weakly positive relationship in the high light treatment. It is less common to predict final mass by correcting for initial size differences. We are essentially examining what the competitive outcome, but only for the end point of this experiment, would be if there were not initial size differences.

In some species - treatment combinations there was a negative relationship between final mass and initial size. Each species treatment combination was only

replicated with 5 individuals; hence these relationships may be driven by noise in the data as opposed to a true negative relationship. However, the separate initial size - total mass relationships for each species-treatment combination are still used in the size correction as they greatly improve the AIC of the model, and may contribute to the inaccurate predictions. Both of these issues emphasize how the RGR method is a 'get-around' for dealing with the difficulty of size dependant growth rate measures. The problem is not addressed directly, hence in some situations the metric operates well, while in others in can create spurious or incorrect results.

Size correcting for final mass resulted in more problems than for the other methods. It makes much more sense theoretically to use this method for RGR but these problems do highlight how the method is a get-around for the size dependency of growth and does not really address how plants grow. Essentially this all comes down to the size dependency of growth, this method gets round some of the undesired effects in the analysis, but really we need a method of analysing growth that takes account of this size dependency in a mechanistic or functional fashion, rather than using metrics purely because they enable us to use linear models because of there simplicity in implementation.

We have discussed a number of caveats of this method of accounting for initial size differences, including predicting off the regression. However we feel that the biggest draw-back is that growth metrics such as AGR and RGR designed for use with linear model analysis assume linear or exponential growth while this is unlikely to be the case; in reality it is probably somewhere in-between such as proposed by Enquist et al. (1999).

There are many ecological papers in the current literature that use RGR as their main method of quantifying differences between performance, and some even reference Hunt's (1990) work, e.g. (Brearley et al. 2007) – yet few acknowledge the pitfalls or assumptions that he carefully described – such as RGR assuming exponential growth. However, in some cases researchers do attempt to address the assumption that RGR, assumes exponential growth. Often the presence of exponential growth is examined by plotting log mass against time and assessed by eye – If there is a straight line rather than an asymptotic curve then the conclusion is that growth is linear. Using this method Swanborough and Westoby (1996) believed that they had exponential growth from days 6 to days 40, and thus data suitable for analysis using RGR. In some papers researchers also plot ln dry mass against time yet use a formal statistical test for non-linearity (Wright and Westoby 1999). Angert et al. (2007), used a combination of testing linearity by eye, and supported this by a lack of need for quadratic or cubic terms in their model. However it can be very difficult to distinguish deviations from linearity either by eye or using null hypothesis significance testing and thus is not easy to detect with the above methods. It is possible to be growing in a sub-exponentially (Pacala et al. 1994) and yet be only a small section of an asymptotic curve. (figure A, in introduction). As we suggested in the introduction, a section of an asymptotic curve could look linear. The difficulty to see the curvature of a line by eye will be exasperated by calculating species means and adding error bars as Swanborough and Westoby (1996) have done, rather than plotting all the data points. If they were truly growing exponentially then adding size into their model would not have an effect, suggesting that the here small seeded species truly do grow faster. It would be very interesting to test this with this dataset and establish whether the problem we have found is present in other plant forms and systems.

Blackman (1919) first introduced RGR as a similar concept to compound interest in banking. The problem with the analogy is that plants need to allocate their resources to different areas whereas with banking you don't need a proportion of the funds to support the funds gaining interest – all of the funds gain interest. However for a plant, particularly a tree, a substantial amount of the plants mass is required to support the leaves, yet the stem cannot directly photo-synthesise itself.

There are a number of reasons proposed for why growth may slow with an increase in plant size; it could be due to differences in leaves either physiological or structural; structural constraints throughout the whole plant, such as an increased requirement for stem support; or growth may slow due to changes in resource allocation throughout ontogeny. Whatever the reason we need analysis of plant growth to reflect this slowing of growth if we are ever going to elucidate the intricacies of ecological trade-offs.

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Chapter 3

Mechanistic plant growth models: Getting to the biology behind plant growth

With Andrew Hector, Toby Marthews, Drew Purves and Lindsay Turnbull

Abstract

In chapter 1 and 2 we used linear models to analyse growth. This is extremely constrained because growth is not a linear process. Conventional functional plant growth analysis – using non-linear regressions - involves fitting a series of biomass data to a growth curve of which the shape is set independently of the data. This is still constrained as using a particular curve dictates that the parameters are determined by mathematical boundaries rather than biological interpretability. In this chapter we develop a more mechanistic plant growth model for our dipterocarp growth data using a series of difference equations to describe the process of growth.

We attempt an exhaustive process of model fitting, comparing 8 different functional forms of growth. We fit a full stepwise regression for each growth form, resulting in many separate models. We present our process of model selection using information criteria graphically.

The models fit the data poorly, perhaps because the species-specific parameters allow too much flexibility in the model. For this reason we then fit one fully specific hierarchical model, where the species-specific parameters are shrunken towards the grand mean. This model fit is also not as optimal as we would have hoped. We test the fit of the model using cross validation, which suggest that this model is not optimal. We explore the possibilities of why our model is inadequate in explaining the data. Essentially the growth process in our system of differential equations is too simplistic to model the plant growth behaviour in our dataset. This is probably mainly because our model is constrained to positive growth.

We conclude this chapter with a theoretical exploration of possible plant growth strategies and suggest which approach we feel may be the best to fit to this data.

Section 1

A renewed framework for plant growth models

Simple linear model models such as linear regression and ANOVA constrains the analysis of growth which is not necessarily a linear process. Using non-linear regression (another conventional approach) involves fitting a pre-determined growth curve (for example changes in the lower part of the curve may affect an asymptote). However, this is still constrained in that the parameters describe the shape of a curve rather than the biology of growth. In addition, the analytical methods of implementation usually require a closed form solution, which can limit the creation of a more biologically informative analysis. A mechanistic model enables the fitting of a biologically informative growth curve by using a series of differential equations that describe the physiology of growth.

A pioneering paper by Turnbull et al. (2008) used a mechanistic model to analyse the growth of sand dune annuals. They overcame the size dependency of RGR in two key ways; firstly by allowing their main growth parameter (G) to be driven by leaf rather than total biomass – which more accurately reflects the process of growth; and secondly, by incorporating a biomass triggered switch allowing the slowing of growth from exponential to linear.

In addition to these two crucial differences, Turnbull et al.'s (2008) growth model predicts daily size changes depending on environmental conditions. In their case, response to temperature and day length addressing the issue that species experience different environmental conditions at different sizes. Essentially growing the plant in steps of 1 day allows the model to predict daily size changes depending on the current environmental conditions and provides the opportunity to estimate the intrinsic growth-potential of a species independently of the environmental conditions. The estimated physiological growth potential of different species can be compared through the size independent growth rate parameter – G. Additionally the model simultaneously allocates carbon to above-ground and below-ground compartments in daily time steps, and allows for periods of tissue loss – through time varying covariates - which would not be possible with a conventional functional growth curve.

In essence, the framework that Turnbull et al. (2008) presented more accurately reflects the biology of plant growth, and thus, is much more likely to provide results which are easily interpretable, interesting and ecological accurate.

Resurrection of a theoretical model

In actual fact the idea of allocation of biomass to compartments used in the analysis of Turnbull et al. (2008) is not completely new, and have been discussed in Tilman's (1988) monograph. Tilman (1988) proposed a mechanistic plant growth model called 'ALLOCATE'. He put forward that the rate of growth is determined by a pattern of allocation to roots, stems, leaves and seeds.

Tilman (1988) emphasised the differences in allocation strategies of plants by comparing an alga – which essentially allocates all of it's resources to production and can achieve very high growth rates – to a huge *Sequoia dendron* tree - that allocates a huge amount of its carbon away from photosynthetic material (leaves) to structure (tree trunk). He hypothesised that any allocation away from photosynthetic material would result in a reduction in growth rate. Tilman (1988) believed that when a plant is not limited by any resources, growth could be an exponential process through the continual reinvestment to production. In our view, this idea reinforces the hypothesis that plants that invest away from photosynthesis to structural material cannot grow exponentially and an ideal way to deal with this is to directly account for allocation within the growth analysis.

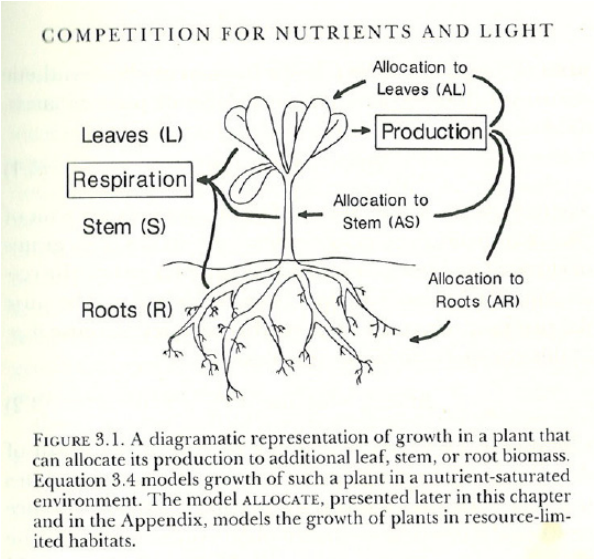

FIGURE 3.1. A diagramatic representation of growth in a plant that can allocate its production to additional leaf, stem, or root biomass. Equation 3.4 models growth of such a plant in a nutrient-saturated environment. The model ALLOCATE, presented later in this chapter and in the Appendix, models the growth of plants in resource-limited habitats.

Figure 1: Tillman's graphical representation of the allocate model (with original legend)

Figure 1 graphically represents Tillman's ALLOCATE model: a simplified framework of growth. While each of the compartments of the plant - leaves, stem and roots - respire only the leaves are directly responsible for photosynthesis and therefore production. The carbon fixed created through the process of photosynthesis can then be allocated to each of the compartments. While Turnbull et al. (2008) didn't directly draw on this theoretical framework, the mechanistic model that they employed and fitted to their data adopts a very similar approach. This method is highly refreshing in its simplicity whilst reflecting what we understand about the process of plant growth very well.

Section 2: The process of model fitting

2.1: The system of differential equations: A three compartment daily growth model.

Using the model of Turnbull et al. (2008) as a framework, we set about adapting the model to our dipterocarp seedling dataset. One key difference of our dataset is that we do not have time-varying covariates of environmental data, as the climate in this area is largely aseasonal; in addition the seedlings were watered daily, eliminating any effects of short droughts or drier months. The other major difference is that their model has two compartments where carbon is allocated: to above ground biomass and below ground biomass. For our dipterocarp seedlings there were 3 distinct compartments; leaves; stem and roots. However in essence, we fitted a very similar growth model.

The daily plant growth model is represented through a sequence of discrete time (see box 1 for details on discrete time versus continuous time) difference equations below. For a given plant species i and time d (days after the start of the experiment) the mass of each compartment (shoot, leaf and root) is determined by a daily mass increment plus the mass of that compartment on the previous day (1a – 1c):

$$M_{i,d+1}^{(leaf)} = M_{i,d}^{(leaf)} + \Delta M_{i,d}^{(leaf)} \quad (1a)$$

$$M_{i,d+1}^{(root)} = M_{i,d}^{(root)} + \Delta M_{i,d}^{(root)} \quad (1b)$$

$$M_{i,d+1}^{(shoot)} = M_{i,d}^{(shoot)} + \Delta M_{i,d}^{(shoot)} \quad (1c)$$

The fraction of carbon allocated to the shoot, leaf and root compartments are governed by parameters F1 and F2:

$$\Delta M_{i,d}^{(shoot)} = F1_{i,d}.P_{i,d} \tag{2a}$$

$$\Delta M_{i,d}^{(leaf)} = (1-F1_{i,d}).F2_{i,d}P_{i,d} \tag{2b}$$

$$\Delta M_{i,d}^{(root)} = (1-F1_{i,d}).(1-F2_{i,d})P_{i,d} \tag{2c}$$

where F1 divides the carbon gain between the stem compartment and a combination of the leaf and root compartments. The parameter F2 divides the carbon gain between the leaf and the root compartments.

The effect of size on carbon gain in our initial model – as per Turnbull et al. (2008) – is based around a switch from exponential growth to linear growth:

$$C_{i,d} = \begin{cases} G_{.i}.M_{leaf} & \text{if } M_{leaf} < M_{ref} \\ G_{.i} & \text{if } M_{leaf} \geq M_{ref} \end{cases} \tag{3}$$

We incorporate this in the model by initially having total carbon gain (C) proportional to leaf biomass - and thus growth is exponential. The switch to linear growth occurs when the leaf biomass reaches a critical reference mass, M_{ref}, which is one of the parameters of the model. After this switch total carbon gain (C) is independent of leaf biomass and is therefore linear – entirely determined by G:

$$G_i = G_{\text{int}} + G_{slope}.(light - 0.03) \tag{4}$$

G determines the growth in response to light in a way similar to a normal linear regression. We normalised light so that the intercept – G_{int} indicates the growth rate in the lowest light treatment rather than in zero light. G_{slope} is the growth response to light.

The fraction of biomass allocated to each of the compartments is determined by:

$$F1_{i,d} = 1/[1+\exp(-A1_{i,d})] \quad (5a)$$

$$F2_{i,d} = 1/[1+\exp(-A2_{i,d})] \quad (5b)$$

$$A1_{i,d} = \gamma 1 + \gamma L1(light - 0.03) \quad (6a)$$

$$A2_{i,d} = \gamma 2 + \gamma L2(light - 0.03) \quad (6b)$$

Equation 5a and 5b are logit functions bounding F1 and F2 between 0 and 1. Equation 5a and 5b are linear functions to set the effects of biomass via $\gamma 1$ and $\gamma 2$, and light via LL1 and LL2, on allocation.

2.2: The functional forms of growth

In the Turnbull et al. (2008) model the functional form began with exponential growth and was allowed to switch to linear growth in response to a reference mass – one of the parameters of their model (equation 3). While the reference mass parameter was species specific, all of the species switched their growth from exponential to linear at a similar mass. This indicated that there was a true slowing of growth in this dataset, independent of the slowing of growth brought about by allocation. Their explanation was that this explained a slowing of growth in response to pot constraints – and this would fit with all species slowing their growth at a common mass.

It would have been possible that the model fitting algorithm could have found that pure linear growth or that pure exponential growth fitted best to the data. In these cases the reference mass parameter would have been determined to be smaller than

the lowest value of mass in the data – indicating pure linear growth; or larger than the largest mass value in the data – indicating pure exponential growth.

This system of a switch in the type of growth is unusual and may be difficult for many readers to understand. We felt that, as many researchers are familiar with linear and exponential growth from other studies and analysis – it would be interesting to also fit models using the same daily allocation framework, but constraining growth to be either linear or exponential. This would enable us, and readers, to compare these different functional forms of growth, evaluate which are most likely, and assess how assuming different forms of growth at the beginning of an analysis may influence our results and understanding of the data. Here, in addition to linear, exponential and an exponential-linear switch growth models we briefly describe 5 other functional forms and our justification for using them.

Enquist et al. (1999) argued that growth always scales with mass to the power ¾ - if this were true a power-law model with a power of 0.75 should explain plant growth best. There is much debate about the justification of their assumptions (Coomes 2006) so we included a power-law growth model with the power as a free parameter in order to test this prediction. We are interested in testing how our framework fits to tree growth data when the functional form is fixed to a power-law as per the WBE model. Do we also find that Mass growth scales to 3/4? Does this affect our understanding of small scale processes and trade-off's based around individual plant growth?

As there is nearly always allocation away from photosynthetic material to other compartments of a plant such as structural tissue it is perhaps theoretically impossible for growth to be truly exponential. However, in addition there could still be a sudden slowing of growth due to pot constraints – so we also included a model

which starts off with power-law growth – with beta a parameter of the model – and also allows a switch to linear growth.

While Turnbull et al. (2008) allowed a switch from exponential growth to linear growth, we felt that in the case of seedlings that are continually allocating a large proportion of biomass to structure, that their growth rate would continue to decline indefinitely. The problem with a hard switch from exponential to linear growth is that we may constrain growth to be a mixture of the extremes when in fact it is always somewhere in between. We therefore proposed in addition, two functional forms that had a gradual decline of beta from exponential to linear. The power was never at either of the extremes but declined 1. exponentially and a 2. faster than this. One advantage is the added simplification that there is no switch and thus no 'if' statement in the formulae, however the formula is slightly more complicated. These models have been termed phimart1, and phimart2.

The Gmax model is theoretically quite different. Here the plant grows exponentially until it hits its maximum possible growth rate that it can achieve and then levels off with this growth rate. In this case the reference for the switch is actually a reference growth rate rather than a mass. Each of the functional forms of growth are presented graphically in Figure 2, and mathematically in box 2. Note Gmax is not included in the graphical representation.

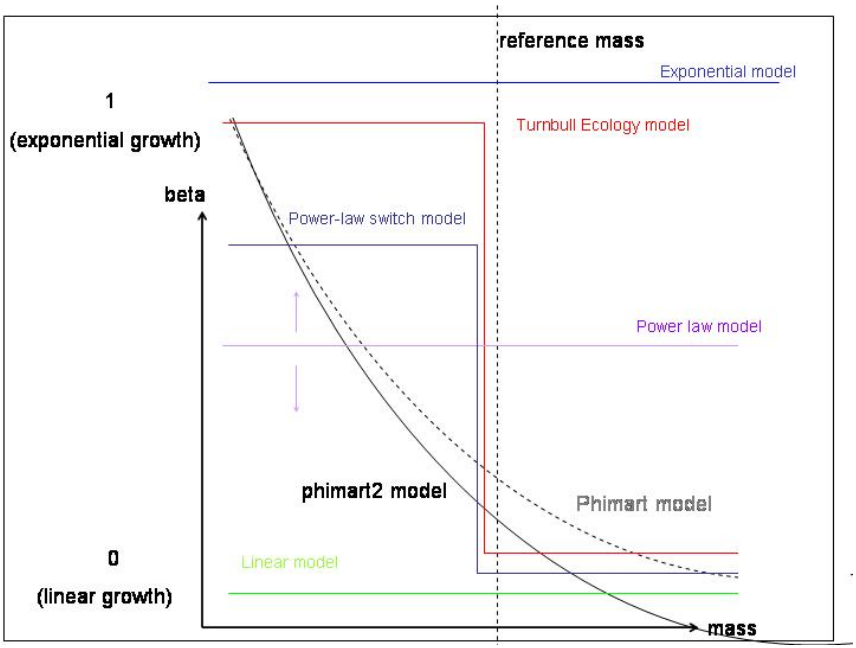

Figure 2: The change in exponent from exponential to linear growth in the different growth forms.

Beta – The exponent indicating the extent to which mass is multiplying during growth. In pure exponential growth this is 1 (light blue line). In pure linear growth this is 0 (green line). In power-law growth the exponent could be anything, but is most likely to be between 0 and 1 such as proposed by Enquist (0.75) (purple line). In the Turnbull model, growth switches from exponential to linear (red line), in response to a certain reference mass. In the power-law-switch model the exponent switches from power-law growth to liner growth (dark blue line). PhiMart1: exponential decay of beta in one way (black line). PhiMart2: Gradual decay of beta in another way (dotted black line). Note lines are jittered to avoid overlap.

Box 1: Formulas for functional forms

Linear: $C_{i,d} = G.B_d$

Exponential: $P_{i,d} = G.B_d.M_{leaf}$

Power-law: $P_{i,d} = G.B_d.M_{leaf}^{\beta}$

'Turnbull' Exponential-linear switch:

$$P_{i,d} = \begin{cases} G.B_d.M_{leaf} & \text{if } M_{leaf} < M_{ref} \\ G.B_d.M_{ref} & \text{if } M_{leaf} \geq M_{ref} \end{cases}$$

Power law linear switch:

$$P_{i,d} = \begin{cases} G.B_d.M_{leaf}^{\beta} & \text{if } M_{leaf} < M_{ref} \\ G.B_d.M_{ref}^{\beta} & \text{if } M_{leaf} \geq M_{ref} \end{cases}$$

"PhiMart" : $P_{i,d} = G.B_d.M_{leaf}^{\exp(-M_{leaf})}$

"PhiMart2" : $P_{i,d} = G.B_d.M_{leaf}^{\exp(-M_{leaf}^2)}$

GMAX

$$P_{i,d} = \begin{cases} G.B_d.M_{leaf} & \text{if } P_{i,d} < G_{\max} \\ G_{\max} & \text{if } P_{i,d} \geq G_{\max} \end{cases}$$

Parameter estimation for the daily growth model

Likelihood analysis

The likelihood analysis used the destructive and non-destructive measurements of the three plant compartments in each of the light treatments to estimate the parameters in the daily growth model (Box 1). The first step is to define the log-likelihood of the data, given the model and the parameter values. The analysis proceeds by finding the parameters that maximise the log-likelihood of the data and the confidence intervals on each parameter. The main parameters (G_{int}, G_{slope}, M_{ref},

F1, F2, γ1, γ2, LL1, LL2 and for some functional forms β) govern the behaviour of the daily growth model. The other 3 parameters (ρ_{stem}, ρ_{leaf} ρ_{root}) set the magnitude of the individual-to-individual variation in biomass, which was required to define the error distributions. The key assumptions are that each observation comes from a normal distribution and that the observations are independent. The analysis shares these features with standard non-linear least-squares regression carried out on log-transformed data.

General details of the algorithm

Markov Chain Monte Carlo (MCMC) has its beginnings in a paper by Metropolis et al. (1953) and Hastings (1970), although the idea was not taken up until much later. MCMC methods are now widely implemented in biological sciences as the high levels of computational power required are now available. MCMC methods are algorithms for sampling distributions including many of the random walk algorithms. They are often described as a random walk in parameter space. Because they are unintelligent in this way they can take a very long time to converge. One such random walk is the "Metropolis-Hastings" algorithm which requires a symmetric jump distribution and uses a method for rejecting proposed moves (Clark 2007).

Intuitively the algorithm accepts any change in the parameter values that increases the log likelihood. However, perhaps counter intuitively the algorithm also has the ability to accept changes in parameter values that decrease the log likelihood. This is important in complex non-linear problems such as these because it allows the

algorithm to escape from local maxima – or small peaks in the parameter space – and find the global maximum or 'true' value of the parameters. In order to obtain the confidence intervals a list of the parameter values that are output from the MCMC scheme are ranked, the tails removed and the range indicated the confidence intervals. The MCMC sampling scheme used in this exercise has previously been found to be effective at finding MLEs and confidence intervals e.g. (Purves et al. 2007a, Purves et al. 2007b, Turnbull et al. 2008). The details here have been directly adapted and summarized from Appendix B in Turnbull et al. (2008); see their appendix for a full description. In our model, the first model data comparison is at the second harvest – see box 3 for further details and for alternative methods.

3.1: Stepwise regression process

When possible, it is arguably desirable to fit all possible combinations of models (Roeder 2008). However, there are counter arguments and this can quickly lead to thousands of models - for example, not including the 3 noise parameters, our model has 11 parameters, in order to run every possible combination there would be 2^{11} = 2048 models or even tens of thousands of models if the 3 noise parameters were included there is a total of 14 parameters 2^{14} = 16384 models. While choosing a 'best' model with maximum likelihood or information criteria such as AIC or BIC may be possible, evaluation of goodness of fit or checking how sensible the parameter estimates are is near on impossible with this many models. For this reason we used a stepwise regression loop that initially runs the model with all parameters shared between all species (global) and then runs the model with each of the parameters varying for each species (species specific). The algorithm then evaluates which parameter had the greatest effect on improving the fit of the model – assessed by maximum likelihood. This parameter is then fixed to be species specific and the

algorithm then re-runs the loop with each of the remaining parameters fixed as species specific. This meant that the number of parameters to be estimated (not including the error parameters) varied from 8 (all parameters global) to 108 (all parameters species-specific). This method has the additional advantage that we can also asses the importance of each parameter by how early in the regression loop it has been set to species specific.

Box 2: Discrete time versus continuous time formulation:

The formulas for growth curves can be written using a continuous time formulation (using a differential equation) or in a discrete time formulation (using a difference equation). It is important to recognise that these are not just different ways of writing (or visualizing) the same formulae, but a different formulation that will often (but not always) result in a slightly different solution. Both formulations are used in the literature, and the choice will often depend on the nature of the data and sampling intervals. In Turnbull et al. (2008) and in our variation of the model described here we use a discrete time formulations. This is because we feel the diurnal cycle of growth imposes a timestep on the physiological processes involved. The model is truly discrete in time and working through daily jumps in plant masses and allocation. Here we present the different formulations for the simple example of linear growth. In this case the analytical solution is the same for both formulations, although this is unusual.

Example for Linear Growth	Continuous time formulation (a **differential equation, DE**)	Discrete time formulation (a **recurrence** or **map**)
	$\frac{dM}{dt} = a$	$M_{t+1} = M_t + a$
Analytical solution or **Integral**; in this case is the same for both formulations	$M = M_0 + at$	

Box 3: Initial size parameters versus fixed mean start values:

There are two distinctly different ways of evaluating the start conditions for the model. In our case we harvested larger numbers of seedlings at the beginning of the experiment in order to get the best possible estimate of biomass for the starting conditions. We then fixed the initial size to the mean of these biomass values. The first harvest in therefore only used for the setting of the initial starting condition. There first model-data comparison would therefore be for the second harvest. The disadvantage of this method is that with a large amount of plant to plant variation and low samples sizes it is possible that the observation mean is far away from the true mean. In this case we would be fixing the initial size at the observation mean, which may affect the rest of the model fitting.

An alternative approach would be to have the initial start condition as a free parameter. This would involve estimating an initial start size for each compartment of each species. One big disadvantage of this method is that it adds additional parameters to the model. As we would expect the initial destructive harvest to give a very good estimate of starting size, and it reduces the number of free parameters in the model, we chose to fix the initial starting conditions, and evaluate the model using the subsequent harvests.

Noise parameters

Mixed effects models (or hierarchical models) have multiple error terms representing noise at different levels of the data set. In this approach we have 3 error parameters; one for each compartment of each plant species. Due to different sampling techniques we would expect the noise to be different around each of these parameters. As some of the species would be much more variable than others it makes sense to keep these 3 noise parameters species specific. If they were not always species specific the algorithm may select one of the noise parameters to be species specific early on in the model fitting process. Keeping the noise parameters species specific means that all of our models – with different combinations of global and species-specific parameters are different biologically. See appendix B from Turnbull et al. (2008) for a full description of the likelihood analysis and parameterisation.

3.2: Model fitting and selection

We established which model was fitting best using a combination of the maximum log-likelihood, AIC, and BIC (see figure 2 for an example). We would also examine which parameters became species specific first. Rather than come up with one best model, we tried to understand something about the biology from examining overall which parameters where most important in the model (figure 3).

Development of model formulation for allocation

In our initial model formulation we found no effect of light on allocation in the model, but we did have size dependency of allocation. As the size of individuals varied strongly with the light treatment, there was a danger that the effect of size and light on allocation may be confounded and therefore cause problems with estimation. We tried adding in two additional parameters to allow for allocation to vary with the light treatment as well as with size. In this case allocation was explained almost entirely by light and we therefore removed parameters for the size dependency of allocation.

Average likelihood's versus separate likelihoods per species

Despite having developed the model to a stage where we felt the formulation accurately reflected the way we understood the biology of growth for our data-set; we were still ending up with drastically different likelihoods for some of the similar functional forms. Some functional forms were achieving much higher likelihoods than others despite similar parameter estimates for most of the species. For example the estimate for Int_G was giving similar estimates for most of the species regardless of the functional form. The main difference was pt_beta estimating a negative growth parameter for *Shorea macrophylla* - the largest seeded species (figure 5), and Pt_beta was – as assessed by likelihood – achieving a much better fit.

We realised that a model may improve the fit massively for one species but be less good for all the other species. The vast improvement for one species could

overwhelm the difference in likelihood for the other species As the likelihood is calculated across all the species, we would think that this is a much better model, whereas there is actually only a better fit for one species. In order to identify if this was a contributory factor in our poor model fits we started calculating the likelihood per species.

Through this we discovered that some of the species were preferring a linear fit and some where preferring an exponential fit. As the most flexible functional form pt-beta was therefore fitting the data best overall.

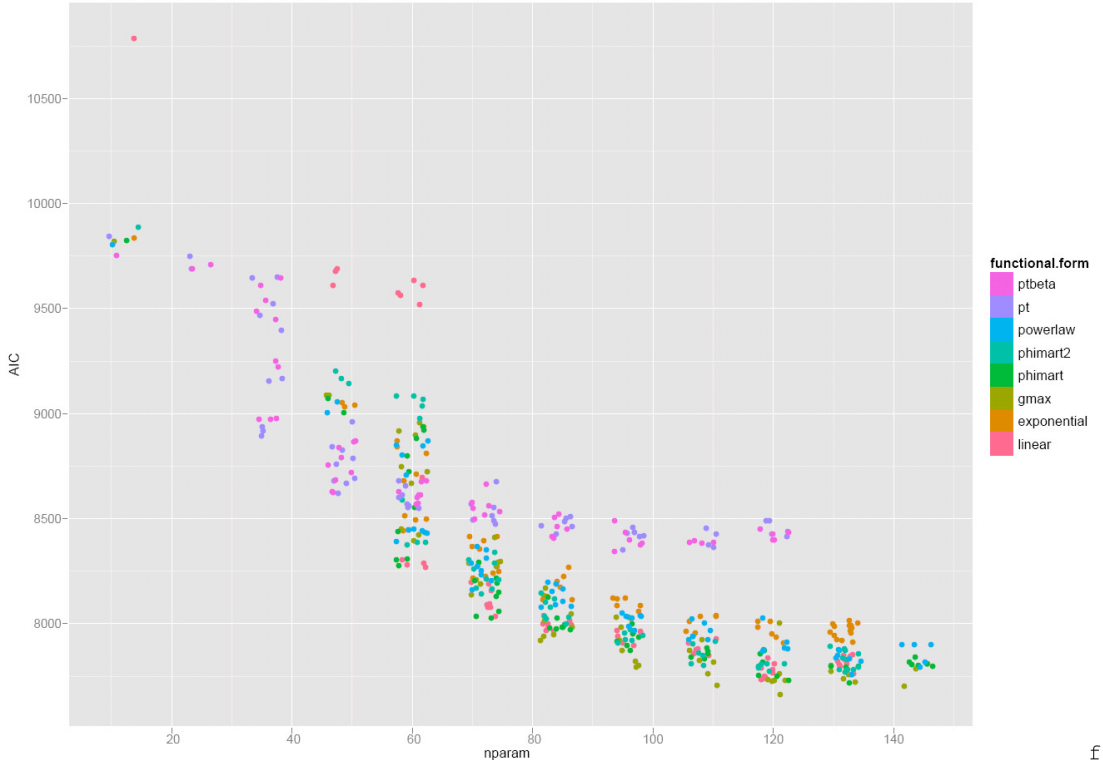
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figure 2

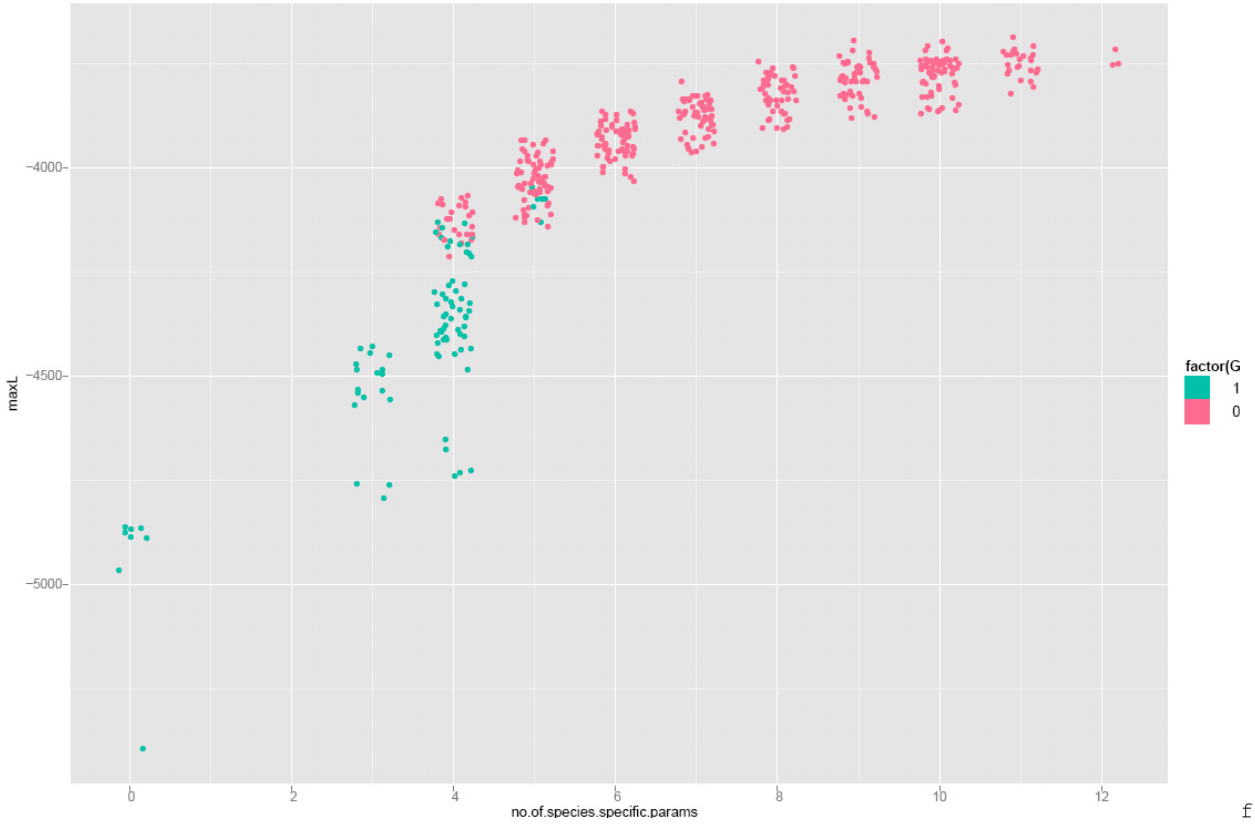
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figure 3

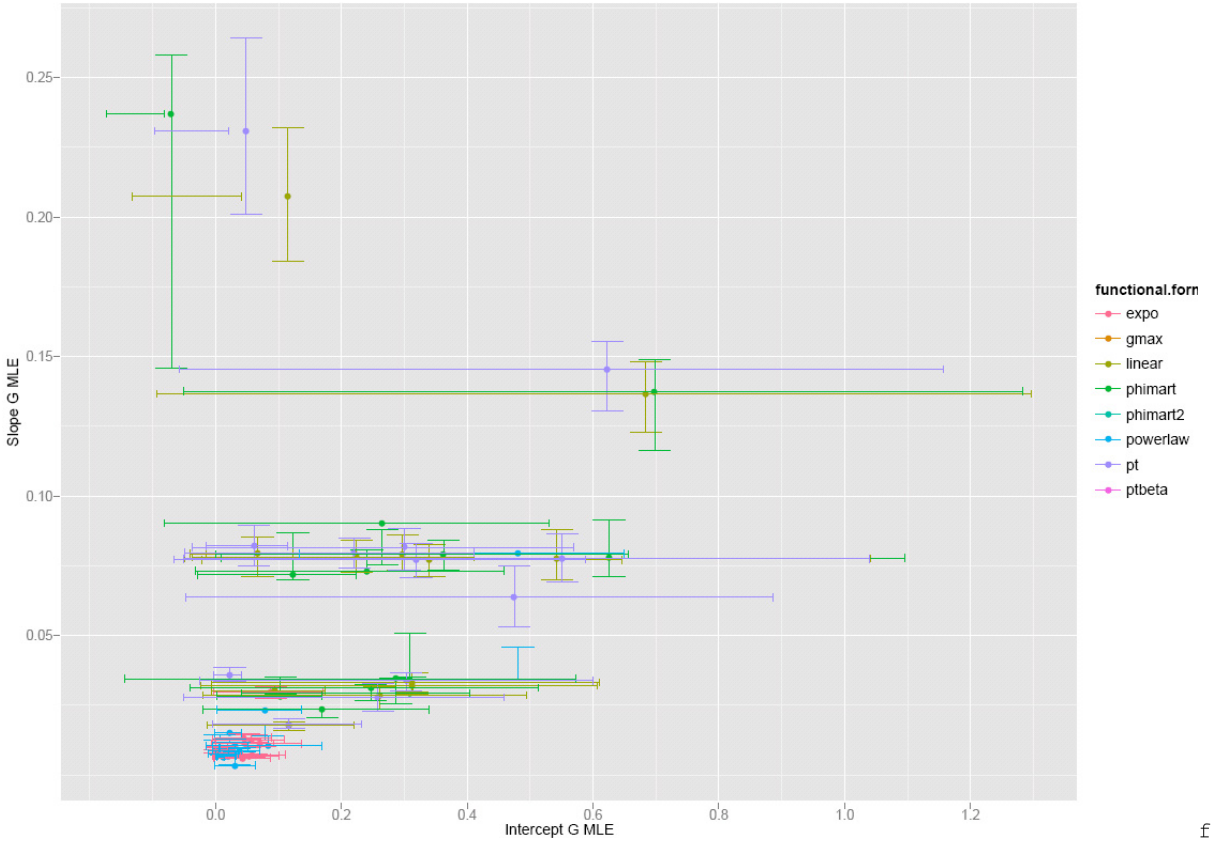
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figure 4

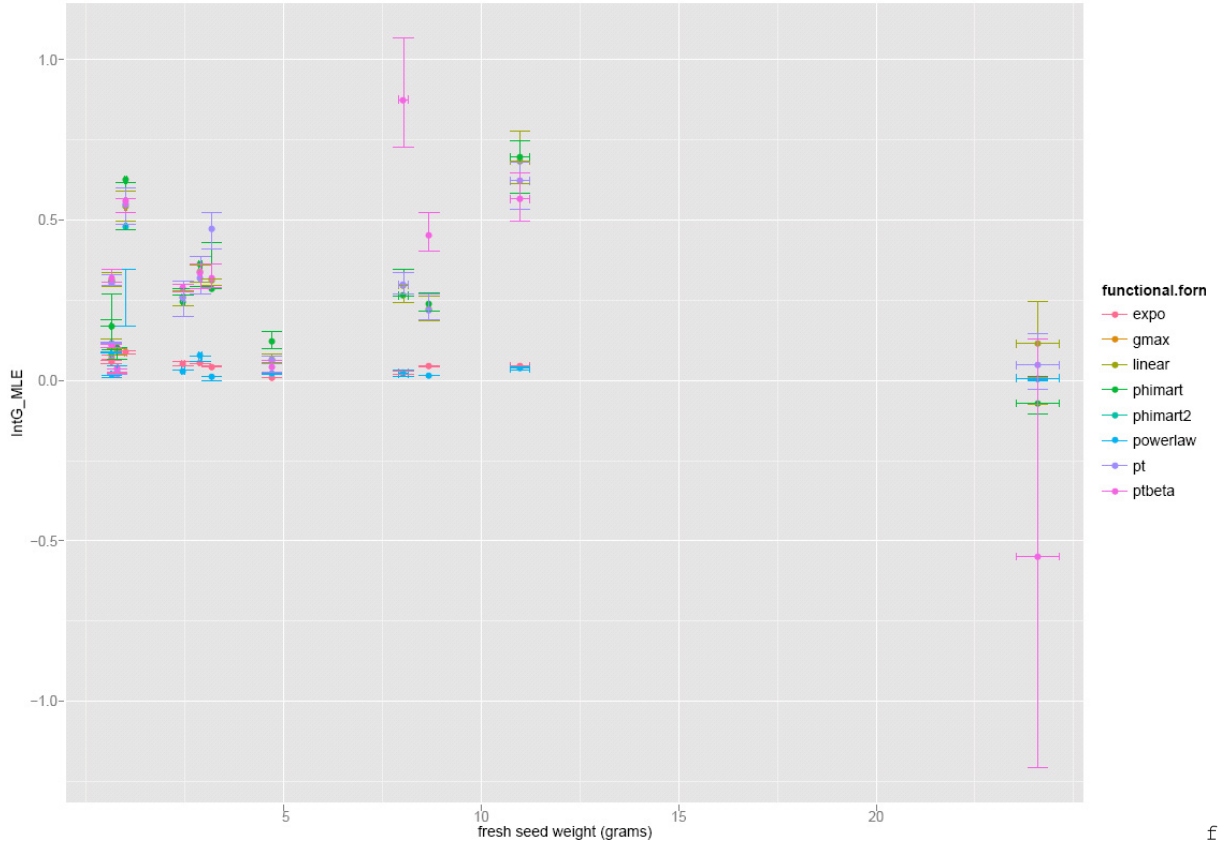
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figure 5

Section 4

Unfortunately many of the adjustments that we had made to the algorithm were making it difficult to fit the model, and when we did manage to fit the model the parameter estimates were poor – for example slope G and intercept G were trading off (figure 4). Often we were finding that the pt-beta model was fitting the best (assessed by likelihood), but it was predicting negative growth rate for the largest seeded species (figure 5). Through calculating separate likelihoods per species we discovered that the flexibility in our pt-beta model was enabling the best overall fit to the data. With the current model formulation, we are effectively fitting a separate model for each of the species. Arguably, this can give too much flexibility to the model and allow the species to differ too much in their behaviours. One way of dealing with this situation is to fit a hierarchical model.

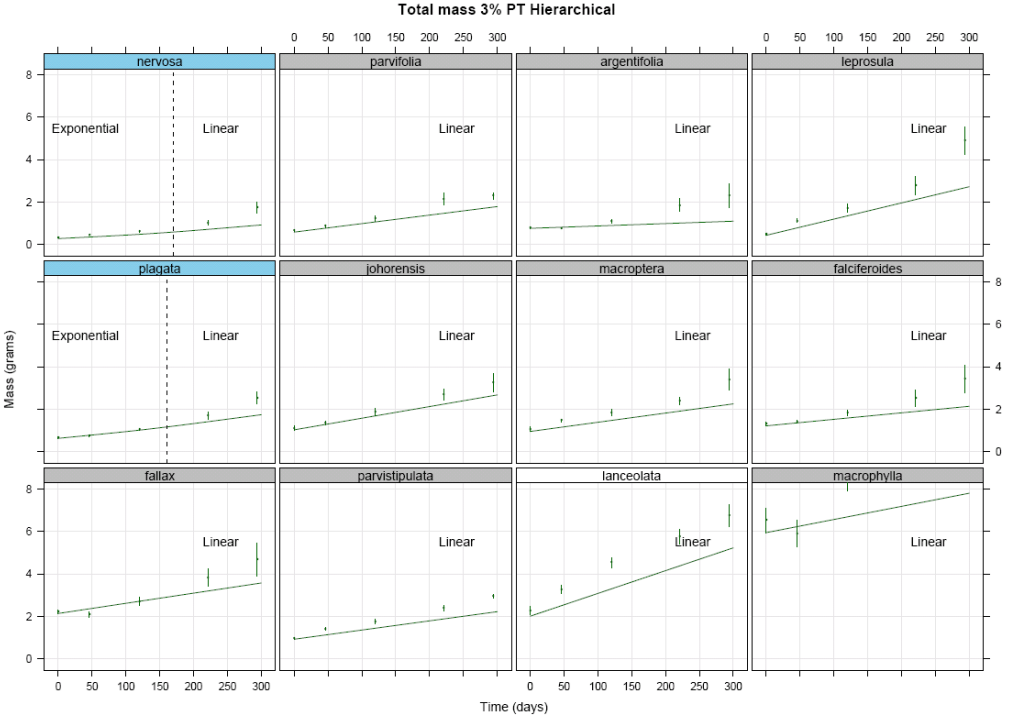
A hierarchical model

We decided that with a large dataset, yet with relatively low replication per species and treatment combination, there was a danger that some very small or large individuals could bring about strange behaviours in the model for some of the species treatment combinations. One solution or approach to attempt to combat this is to fit a hierarchical model. A hierarchical model will shrink the species-specific parameter estimates towards the grand mean, a process often referred to as "shrinkage", see Gelman and Hill (2007). One problem with our attempt to compare many different functional forms was that there were too many models to look at the predicted values from the models and graphically evaluate them against the original data. We

therefore did not try and fit an exhaustive number of models, but chose the one functional form that we thought would fit best – pt – and we fitted a fully species-specific model. We can see in a plot of the predicted values against the raw data (figure 6), that most of the species were growing entirely linearly in the mid-light treatment, but that the two *Hopea's* where growing exponentially for the first half of the experiment. We can also see from figure 6 that for some of the species the model fit matches poorly with the data. The danger is that using the hierarchical model the predictions shrink towards the grand mean too much.

Preferably we would have another similar data set that was not used in the model fitting where we could test the accuracy of our of dataset predictions through independent testing. This was not the case here. The next best thing is to randomly select a subset of the data that is not used for the model fitting - termed "cross validation". This subset can then be used to evaluate the accuracy of the model.

The cross validation suggested that the hierarchical model was in fact shrinking species-specific parameters towards the grand mean too much. We therefore reverted to the previous method of a single level model.


Total mass 3% PT Hierarchical
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argentifolia
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plagata
johorensis
macroptera
falciferoides
fallax
parvistipulata
lanceolata
macrophylla
Exponential
Linear
Mass (grams)
Time (days)

figure 6

Pt-beta fully species-specific model

After having had so many problems with trying to fit a large number of models through the stepwise regression procedure and using a variety of different functional forms, we decided to use only one functional form and to fit a fully species-specific model.

It turned out that having a species-specific Mref meant that all the species grew in either a fully linear or fully power law way (figure 7). One major problem with this is that it makes the size independent growth parameter difficult to compare on an even footing. Whilst this project has gone off on the tangent of the technicalities of analysing growth, the original goal was much more than just obtaining size independent growth rates. Ultimately we would like to investigate evidence for trait evolution with many traits not only growth rates. However we would still need growth rates that are comparable to each other and this model is too flexible to give us these. We can also see from the largest seeded species – *Shorea macrophylla* – that the model is initially predicting biomass values lower than the data and then finishing with predicted biomass values higher than the data (figure 8).

Moreover, it wasn't until we plotted leaf mass per total mass (figure 9) and root mass per total mass (figure 10) that we realised that our model was not capturing some important aspects of the data.

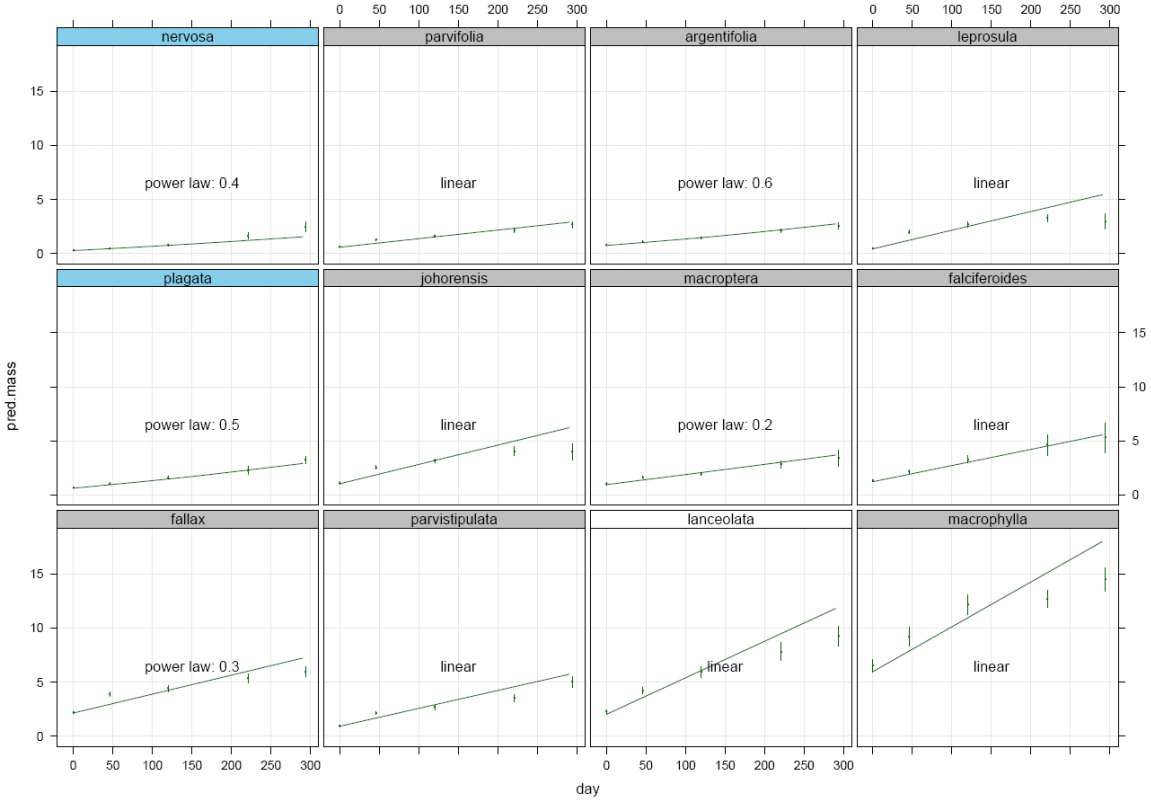
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figure 7

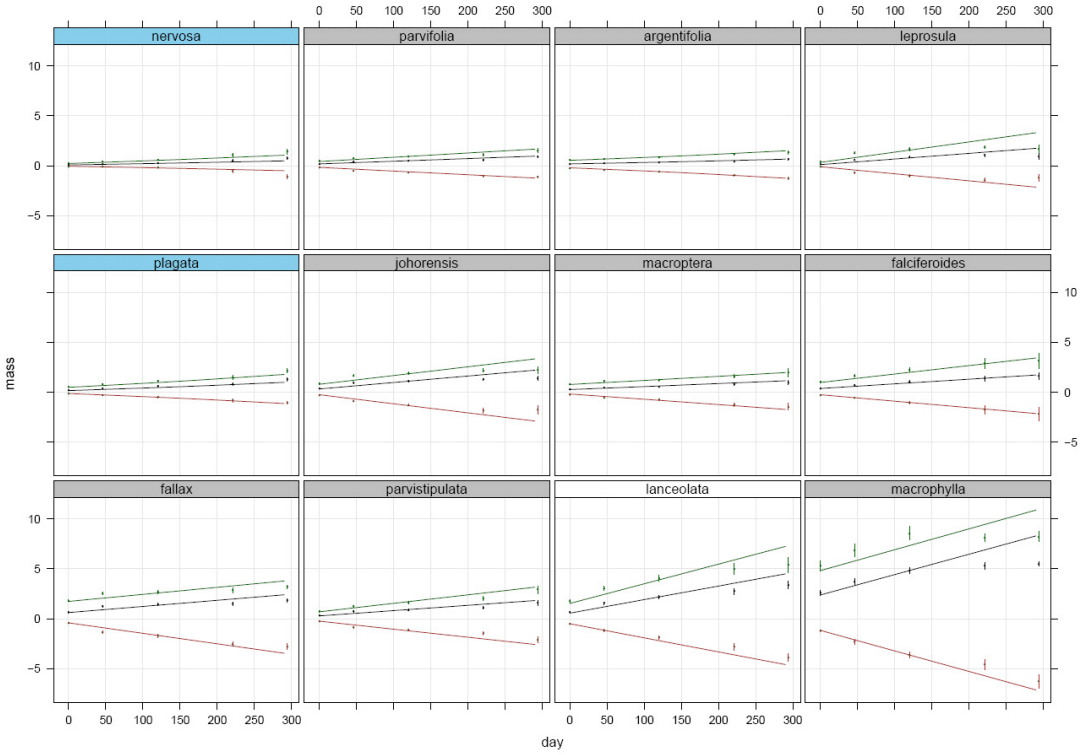
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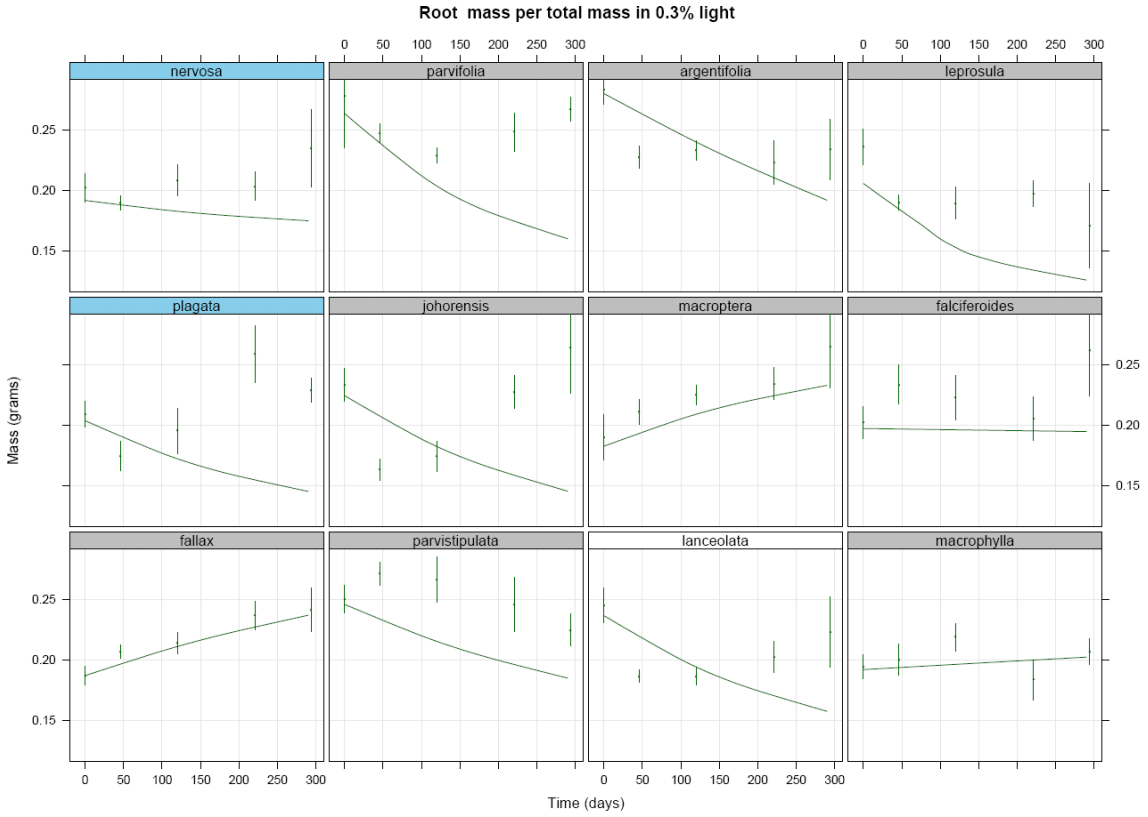
figure 8


Root mass per total mass in 0.3% light
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parvifolia
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leprosula
plagata
johorensis
macroptera
falciferoides
fallax
parvistipulata
lanceolata
macrophylla
Mass (grams)
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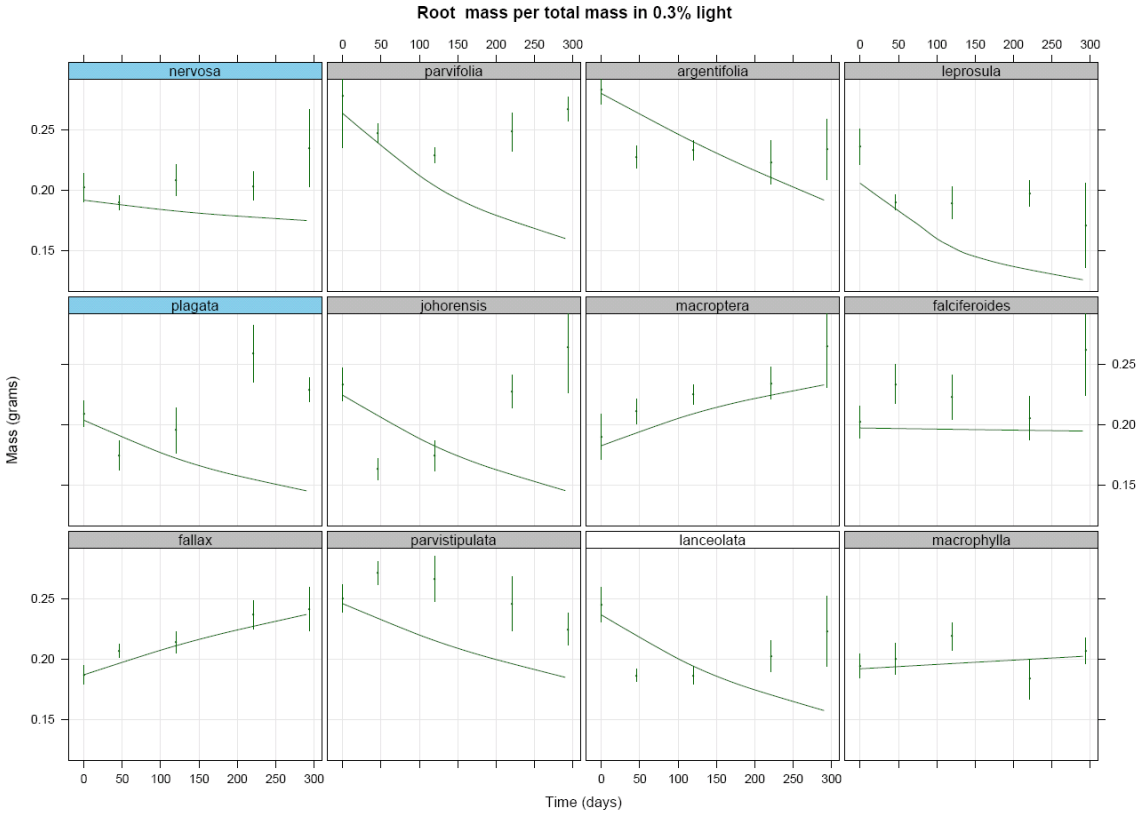
figure 9


Root mass per total mass in 0.3% light
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macroptera
falciferoides
fallax
parvistipulata
lanceolata
macrophylla
Mass (grams)
Time (days)
0.15
0.20
0.25
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50
100
150
200
250
300

figure 10

Future development

Some of the problems we have faced in fitting these models are related to our inexperience with such methods and their technical challenges, however, many of them suggest that the pattern or mechanism of plant growth is more complicated than we have envisaged. It seems that the key to the difficulties is the allocation pattern – which is much more complicated than what we have attempted to model to date (Figure 9 & 10).

Essentially the model that we have tried to fit proposed that plants follow a rule, whereby they allocate according to the current size and environmental conditions. This poorly represents the growth patterns we are seeing. Figures 9 & 10 show strong shifts in the proportion of roots and shoots through time. In addition these shifts are starkly different for the different species and light combinations. We feel that this suggests different plant strategies, and that the dynamics of tropical seedling growth may require some quite different general plant growth models to encapsulate their growth.

A number of different theoretical general plant growth models can be proposed that would help to solve the problems we have encountered. In hindsight these would have been more interesting questions to investigate rather than the functional forms, for example:

1. A rule follower: The plant allocates according to its current size and environmental conditions. For example Turnbull et al. (2008) and the analysis described above.

2. A target plant: The plant has a size-dependant allometry for a given situation and then allocates to get in line with this target plan.

3. A messenger plant: Here parts of the plants (e.g. leaves and roots) send out signals (for example, I'm running out of water), and direct their allocation accordingly. Here the optimal allocation arises from the bottom up.

4. A source/sink plant: Here there are no active rules or decisions. Leaves and roots grow as quickly as they can, but because they depend on each other an optimal allocation arises anyway.

In some ways, it is quite difficult to tell how different these different allocation strategies really are. It could be possible that, ultimately, they may all be able to produce the same eventual behaviour.

A model which is likely to be able to produce quite different growth patterns, and model changes in light environment is a leaf adjustment and replacement model.

5. A leaf adjustment and replacement plant. A tree seedling makes leaves specific to its current light environment. If there light environment changes – either canopy closure or opening in response to a tree fall – or in this case due to placement into an experimental light treatment. By drawing comparisons to a theoretical bacterial growth model this model would propose 3 different types of leaves that can be created. 1. Leaves that are only suitable in their current light environment – if the light environment changes drastically they would be senesced. 2. Leaves that can adapt their physiology to the new light environment – if the environment changes they expend energy adapting and are not productive in this time. 3. Leaves that have an adaptable physiology and are general enough to cope with changes in light environment. A particular plant species could have any combination of these types of

leaves. This set-up would enable short term negative growth rates when environmental variables change resulting in a much more flexible growth model.

Appendix 1: Correct formulation of functional form to normalize G to a 1gram plant rather than an Mref'ed size plant

Linear: $C_{i,d} = G.B_d$

Exponential: $P_{i,d} = G.B_d.\left[M_{leaf} / M_{ref}\right]$

Power-law: $P_{i,d} = G.B_d.\left[M_{leaf} / M_{ref}\right]^{\beta}$

'Turnbull' Exponential-linear switch:

$$P_{i,d} = \begin{cases} G.B_d.\left[M_{leaf} / M_{ref}\right] & \text{if } M_{leaf} < M_{ref} \\ G.B_d & \text{if } M_{leaf} \geq M_{ref} \end{cases}$$

Power law linear switch:

$$P_{i,d} = \begin{cases} G.B_d.\left[M_{leaf} / M_{ref}\right]^{\beta} & \text{if } M_{leaf} < M_{ref} \\ G.B_d & \text{if } M_{leaf} \geq M_{ref} \end{cases}$$

PhiMart : $P_{i,d} = G.B_d.M_{leaf}^{\exp(-\left[M_{leaf} / M_{ref}\right])}$

PhiMart2 : $P_{i,d} = G.B_d.M_{leaf}^{\exp(-\left[M_{leaf} / M_{ref}\right]^2)}$

GMAX

$$P_{i,d} = \begin{cases} G.B_d.\left[M_{leaf} / M_{ref}\right] & \text{if } P_{i,d} < G_{\max} \\ G_{\max} & \text{if } P_{i,d} \geq G_{\max} \end{cases}$$

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Chapter 4

General Discussion on the Size-dependency of Relative Growth Rate

Christopher Philipson

With,

Yann Hautier, Andrew Hector, Cloe Paul-Victor, Drew Purves and Lindsay Turnbull

Abstract

In plants, average relative growth rate (RGR) has been widely found to be negatively related to increasing seed mass. This has generally been taken to infer that smaller-seeded species have physiological adaptations for fast growth. Average RGR is widely used as a plant trait – in the sense that it is thought to indicate something about growth adaptations of different species. Here, we argue that much of what we think we know about species' differences in RGR may reflect nothing more than differences in their sizes. We demonstrate that when growth slows as size increases (a likely universal phenomenon) larger plants will have lower average RGRs even when growth at a given common size is equal. Further, we take 4 groups of plants; species of sand dune annuals, perennial grasses, paleo-tropical tree seedlings and recombinant inbred lines of *Arabidopsis thaliana* – and show that the negative relationship of RGR with increasing seed mass disappears once differences in their sizes are removed. Smaller seeded species may sometimes have intrinsically higher growth but the absence of such a relationship in our size corrected analyses of four widely differing plant groups suggests most negative relationships between RGR and seed mass reported in the literature are driven mainly by differences in plant size.

Introduction

A negative relationship between seed size and average relative growth rate (RGR) has been observed in many different plant growth forms and biomes (Maranon and Grubb 1993, Bloor and Grubb 2003, Rey et al. 2004, Baraloto et al. 2005, Paz et al. 2005, Poorter and Rose 2005, Baraloto and Forget 2007). However, a recent paper by Turnbull et al.(2008) demonstrated for sand dune annuals that this relationship represents size differences rather than physiological differences. It's possible that this is the case for all groups of plants.

Maranon and Grubb (1993), found a negative relationship between seed size and average RGR, as well as a significant decrease in Specific Leaf Area (SLA) as seed size increased – smaller seeded species had thicker leaves, while larger seeded species had thinner leaves. This led Maranon and Grubb (1993) to conclude that the negative relationship between seed size and RGR was due to the thinner leaves of small seeded seedlings, intercepting more light per unit mass, resulting in a greater efficiency of growth. This finding has generally been taken to infer that smaller-seeded species have physiological adaptations for fast growth. However, average RGR does not properly control for differences in plant size.

Diminishing relative growth rates with increased plant size is a widespread pattern amongst vascular plants (Metcalf et al. 2006). Species with larger seeds results in larger plants, and therefore seed size is confounded with plant size. If instantaneous RGR decreases with size and an increase in size, then this will result in a negative correlation between average RGR and seed size. We have demonstrated in a theoretical example how the combination of differences in initial plant size and the slowing of instantaneous relative growth with time overwhelms differences in growth rate detectable by average RGR (Figure 1). As a result this makes real physiological differences difficult to see. Size independent estimates of growth rate – such as the parameter G in Turnbull et al. (2008) – can reveal differences in growth rate that RGR is insensitive to.

Turnbull and Purvis (2008) showed for sand dune annuals, that a negative relationship between seed size and RGR was entirely explained by differences in plant size and biomass allocation, rather than true physiological differences. Therefore, widespread observations of negative relationships between seed size and RGR may be

caused by faster growth of smaller plants rather than smaller-seeded-species having evolved inherently faster growth rates. This is potentially the case for all groups of plants. Here, we investigate whether the negative relationship between seed size and average RGR is explained by initial size differences; using four different plant growth datasets that span three very separate growth forms and two biomes. We show that after accounting for differences in initial plant size there is no negative relationship between RGR seed size.

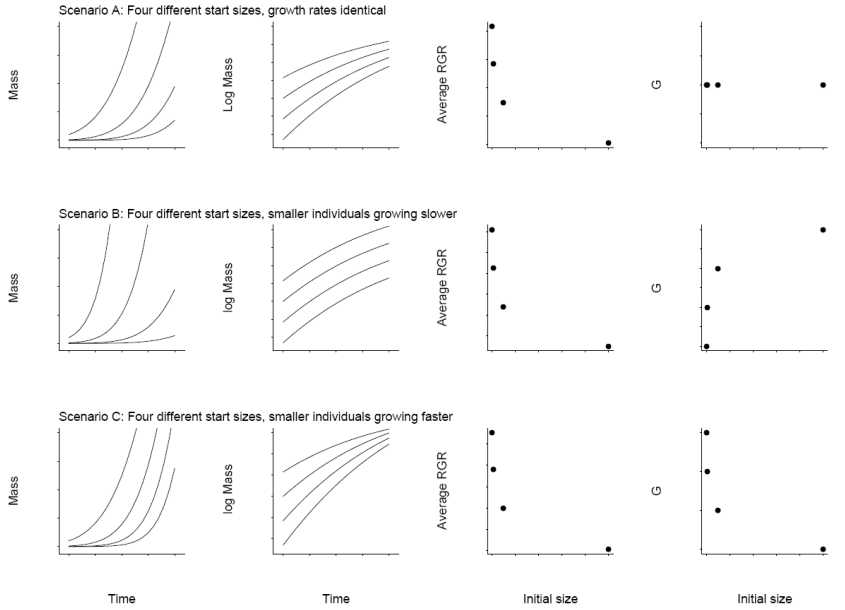

Figure 1 The confounding effects of initial size on the measurement of growth rates.

We present 3 different possible scenarios of the relationships between growth rate and initial plant size. In each case, growth is modelled as a Gompertz function where relative growth slows with increasing size (although the effect will occur with any similar function). For each scenario, we present: mass against time (column 1); log mass against time (column 2); average RGR for each 'plant' against initial size (column 3); and the size independent instantaneous growth rate from the Gompertz function against initial size (column 4). In **(A)**, we have made the parameters of the function (the 'true' growth rate) identical for all species but conventional average RGR measurements (third panel) show that species with smaller initial size have a higher growth rate when assessed with this measure. The size-corrected measure, G, reveals that they are all on the same growth curve with no physiological differences (right hand panel). The differences in RGR arise because species start growing from different initial sizes (note that differences in initial size here are so small as to be hard to see but nonetheless cause RGR to have a negative relationship with initial size in all three cases). In **(B)**, the small-seeded species have higher size-corrected growth rates, G, and in **(C)** the larger-seeded species have higher size-corrected growth rates. Notice that conventional average RGR measures are insensitive to these differences and produce negative relationships in all three cases.

Methods:

A series of growth experiments were conducted for 4 contrasting plant growth forms; grasses (Hautier in prep.) paleo-tropical tree seedlings (chapter 1); Recombinant Inbred Lines of *Arabidopsis thaliana* (Paul-Victor in prep)*;* and sand dune annuals (Turnbull et al. 2008). For each of these different plant growth forms, average RGR's were calculated in the classic way according to Hunt (1990) , with data from an early and late harvest. Average RGR was plotted against seed size for each of growth forms. A simple linear analysis was conducted separately for each data set. Growth rate was then calculated correcting for differences in initial plant size. This was performed in different ways depending on the nature of the dataset. The sand dune annuals were analysed using a daily growth model fitted to multiple harvests, and G – a size independent growth parameter - was estimated (Turnbull et al. 2008). The grasses were analysed using standard non-linear regression with a power-law function - the Amax parameter is comparable between species as a common exponent was used (Hautier et al. in prep). The tropical seedlings where analysed using RGR and a standard linear model, size effects were accounted for by adding initial size as a first term in the model (see Chapter 2). The *A. thaliana* seedlings were analysed using a non-linear asymptotic regression of log biomass data. The growth – or the rate constant - is considered independent of size as the asymptote did not vary with species (Paul-Victor et al., in prep). See Appendix for the methods of each experiment in greater detail.

Results:

All of the groups of plants had a significant negative relationship between Average RGR and seed size (Figure 2). There was no relationship between the size-corrected measures of growth and seed size, regardless of which correction and analytical technique was used (Figure 2).

The grasses had a strong negative relationship between RGR and log seed mass (Figure 2, top-left panel, $F_{1, 7} = 8.6$, $P<0.022$). There was no significant relationship between the size corrected measure of growth and log seed mass (Figure 2, top-right panel, $F_{1, 7} = 1.5$, $P = 0.26$). The dipterocarp seedlings had a negative relationship between RGR and log seed mass (Figure 2, 2nd top-left panel, $F_{1, 10} = 8.5$, $P = 0.015$). There was no relationship between the size corrected measure of growth and log seed mass (Figure 2, 2nd top-right panel, $F_{1, 10} = 2.3$, $P = 0.16$). Within the RIL's of *A. thaliana* there was a marginally significant relationship between RGR and log seed mass (Figure 2, 2nd bottom-left panel, $F_{1, 27} = 2.9$, $P = 0.099$). There was no effect of log seed size on the size corrected measure of growth (Figure 2, 2nd bottom-right panel, $F_{1, 27} = 1.46$, $P = 0.24$). There is a strong negative relationship between annuals RGR and log seed size (Figure 2, bottom-left panel, $F_{1, 6} = 46.7$, $P = 0.000$). The relationship between the size corrected measure of growth and log seed size was strongly positive (Figure 2, bottom-right panel, $F_{1, 7} = 10.43$, $P = 0.000$).

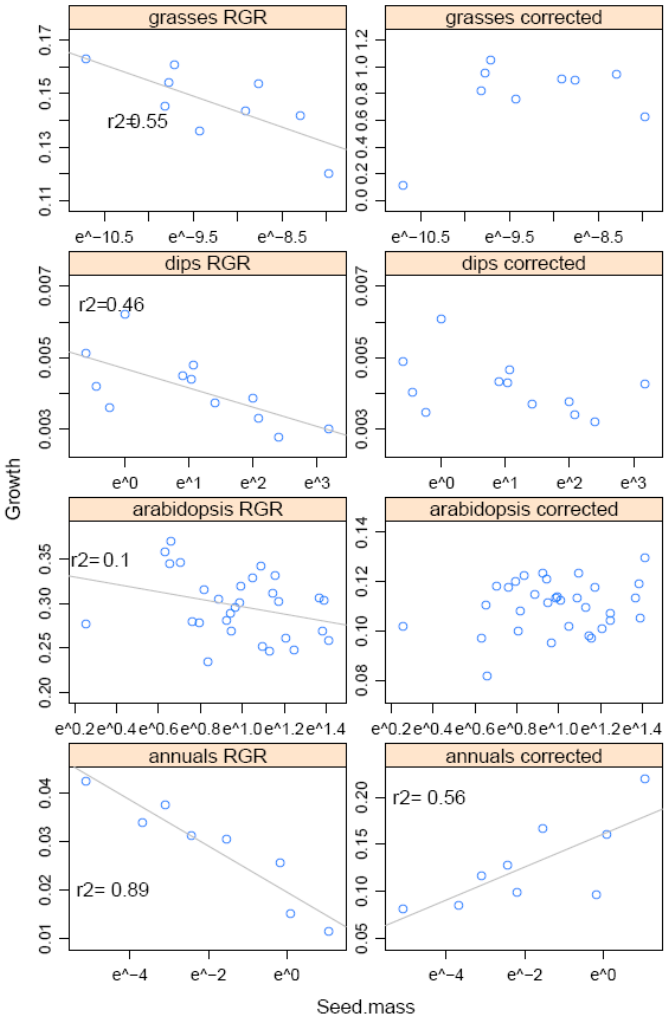

Figure 2: The relationship between average RGR and log seed mass (left) and relationship between size corrected growth and log seed mass (right). Regression lines and R^2 values have been added when there was a significant relationship.

Discussion

As expected from conventional wisdom, all of the groups of plants had a significant negative relationship between RGR and seed size. However, we have demonstrated that once growth measures were corrected for differences in initial size there was no negative relationship with seed size, regardless of which correction and analytical technique was used. This strongly suggests that the negative relationship between seed size and average RGR that we commonly see is driven by differences in initial plant size rather than physiological differences. In fact in the case of the sand dune annuals, the size corrected measure of growth rate was positively related to seed size.

The negative relationship between seed size and RGR has been widely accepted to reflect a physiological adaptation to fast growth in smaller seeded plants in all plant growth forms and biomes, we believe that our analysis and results are strong evidence for a reassessment of this paradigm. In addition, the negative relationship between seed size and RGR is often purported to be stronger in higher light treatments (Bloor and Grubb 2003, Poorter and Rose 2005). It is very likely that the increase in growth rates due to the greater amount of PAR merely accentuates initial size differences and thus the apparent differences in growth rates in relation to seed size. In essence this corroborates our findings that the negative relationship between seed size and RGR is a reflection of size differences rather than physiological differences.

Average RGR is used in many analyses as a plant trait – in the sense that it is thought to indicate something about growth adaptations of different species. For example (Grubb et al. 1996, Leakey et al. 2003, Sack et al. 2003). While this paper only addresses the issue of the relationship between RGR and seed size, there could

be widespread repercussions of the size dependency of RGR that many analysis have not taken into account. The size dependency of RGR particularly affects datasets when there are large differences in size – such as those investigating differences in seed size. There are however, many datasets investigating plant ecology that compare differential responses of species to environmental gradients. If there are considerable differences between species in their initial sizes, this may affect the results and our understanding of the ecology (Massey et al. 2006).

The negative relationship between seed size and RGR is particularly affected by size differences. However, there are many studies of forest ecology that split trees into difference size classes, while this may reduce the problem, there will no doubt still be effects within size classes.

This investigation used four groups of plants; ideally more groups from different ecosystems should be investigated. However, as the datasets we used are from very different groups of plants and areas of the world, it suggests they may be representative of other plant groups and biomes. We believe that average RGR's that haven't been corrected for differences in size should be interpreted with caution, and not necessarily treated as a representation of species growth ability.

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Appendices:

Grasses: Material and methods

Refer to: Hautier in (in prep.)

Nine perennial grass species were selected from those commonly found in European grasslands. The species are: *Agrostis capillaris* L., *Alopecurus pratensis* L., *Anthoxanthum odoratum* L., *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl&C. Presl, *Bromus erectus* Huds., *Dactylis glomerata* L., *Festuca rubra* ssp. *commutata* Gaud. (=*Festuca nigrescens* Lam.), *Holcus lanatus* L. and *Trisetum flavescens* (L.) P. Beauv. These species were grown in pots of 0.6 l containing a mixture of 1:1 of soil (Tref Substrat BF4, gvz_rossat, Otelfingen, Switzerland) and sand (Mauersand, washed, 0-4 mm, Bauhandel AG, Rümland, Switzerland).

Estimation of the growth rate of the nine grass species under three light levels

In April 2006, the experiment was established in order to estimate the growth rate (GR) of each of the nine grass species under different light conditions. The experiment was set up in a compartment of an experimental glasshouse of the University of Zurich (43°23'N, 8°33'E, altitude 549 m a.s.l.). For the nine grass

species, individual seeds were put in pots and left for germination. For each grass species, eight pots were randomly assigned to each of three blocks under three light treatments for a total of 648 plants (9 species x 8 individuals x 3 blocks x 3 light levels). Light treatments included a table with irrigation system and a green shade cloth of different light penetration intensities giving three light levels, control (no shade cloth, 100% light), 42% and 11% light penetration. Plants were harvested nine times at days 7, 14, 21, 28, 42, 56, 70, 83 and 97 after germination. Individuals that did not survive were discarded and the number of plants was adjusted for each harvest so that the amount of plants per harvest and per species was between 1 and 3 for a total of 629 plants. Plants were irrigated automatically on a daily basis. Aboveground plant biomass was measured by clipping plants at the soil level, drying to constant mass in oven and weighing. Additionally, 10 seeds of each grass species were put on filter paper in Petri dishes, and the biomass of emerged shoots was measured by drying to constant mass in oven and weighing. The mean for each species was used as an initial biomass in the calculation of the GR.

The growth rate (GR) was estimated as:

$$\frac{dM}{dt} = \alpha M^{\beta}$$

where M is the plant aboveground biomass of the grass species, t is time, α is an allometric constant and β is the scaling component. From this we derived a power law as:

$$M_t = (M_0^{1-\beta} + \exp(\alpha)(1-\beta)x)^{1/(1-\beta)}$$

where M_0 is the initial biomass of the grass species and x is the time after germination. A nonlinear model was fitted using generalized nonlinear mixed effect

model with the nlme function from the nlme library (Pinheiro & Bates, 2000) for R 2.8.1. (R Development Core Team, 2008) to estimate the values of α and β. The power law and light treatment were treated as fixed effects. α was estimated for each species under each light treatment. Allowing β to vary under each light treatment (likelihood ratio test: **Error! Bookmark not defined.Error! Bookmark not defined.** χ_1^2 = 41.2, P < 0.001) or for each species under each light treatment (**Error! Bookmark not defined.Error! Bookmark not defined.** χ_9^2 = 73.2, P < 0.001) did improve the model but led to overfitting. Overfitting implies that some noise (noninformation) has been included in the structural part of the model and the effects are not part of the actual process under study. Hence, including too many parameters makes the model so specific to the particular data set that they make prediction unreliable. As the goal is to estimate α to make predictions on the performance of *R. alectorolophus* this did not allow β to vary. The model was weighed with a power variance function structure to improve homogeneity of variance of the residuals.

Arabidopsis thaliana: MATERIAL AND METHODS

Refer to: Paul-Victor (2009)

Plant material

Incorporating the natural variation in *Arabidopsis thaliana* permitted a whole-plant partitioning study, using sequential harvests, to characterize growth. 30 RILs were used from a commonly studied RIL population (Cvi x L*er*) (Alonso-Blanco et al. 1998, Alonso-Blanco et al. 1999) and one of its parents, the wild type line Landsberg *erecta*

(L*er*). Three pot sizes were used to provide different degrees of belowground growth restriction. Seed mass information was included and related growth to seed output. The age at inflorescence emergence (bolting) was chosen as the starting point of the reproductive phase rather than the age at first flower for our experiment.

The plant material used for this experiment was the same as the set of lines used in a previous experiment; see Paul-Victor (in prep). This is a set of 30 RILs derived from reciprocal crosses between the two pure lines Landsberg *erecta* (L*er*), obtained as a mutant (*er*) from an accession of northern Europe (Rédei 1962, 1992), and Cvi, an accession from the tropical Cape Verde Islands (Lobin 1983). 30 RILs were grown plus the two parent lines for the experiment described here. These RILs present the main advantage of revealing phenotypes outside of the parental range of variation, thus maximising the range of phenotypic expression (Alonso-Blanco and Koornneef 2000). Using data collected by Alonso-Blanco et al. (1999), this population shows a significant negative relationship ($F_{1,159}$ = 66.9 and p > 0.0001) between flowering time and seed mass with a slope of -0.69 (± 0.046). The 32 lines selected for the experiment are shown among the Alonso-Blanco data see (Paul-Victor in prep).

The lines did not significantly differ from the whole population ($F_{1,157}$ = 2.32 , p = 0.130) with a slope of -0.74 (± 0.075). This negative relationship between flowering time and seed mass illustrates that smaller seeded-lines are sometimes observed to flower later, consistent with the idea that they take longer to extract resources from the pot and hence take longer to reach the inflection point (see *Growth rate rule*). The details about the lines are described fully in the previous experiment (Paul-Victor in prep).

Experimental design

The seeds were obtained from The Arabidopsis Information Resource (TAIR) and one batch of 100 seeds was weighed from each of the 32 selected lines. This is referred to as *sown seed mass*. All seeds were then placed in a cold room at 4 °C for one week to synchronise germination. Plants were grown in small (20 mm diameter), medium (30 mm diameter) and large cylinders (40 mm diameter) inserted into standardized cells (65 mm diameter) within a flat completely filled with a mixture of 50% sand and 50% compost. Each flat contained 35 cells and was 70 mm deep. The cylinders allowed randomisation of pot diameter treatments within flats and ensured that the spacing of individuals in different pot sizes and the surface area available to growing rosettes was exactly the same. However, the three pot sizes provide different degrees of belowground growth restriction. At each harvest there were two replicates of each line and pot size combination.

Pots were sown with four seeds and thinned as soon as seedlings emerged to leave one plant per pot (the most central healthy seedling). The plants were grown in a glasshouse with both natural light and additional artificial lighting which came on automatically when the natural light was below 25 kLux and kept under a cycle of 16 h light (22°C) and 8 h dark (20°C). Germination, bolting (initiation of the flowering stem) and flowering (opening of the first flower) were recorded for each plant to the nearest day. On each day plants were checked for a sign of bolting i.e. flowering stem emergence. This day corresponds most closely with the decision by the plants to initiate reproduction. Bolting age was then calculated as:

$$\text{bolting age} = \text{bolting day} - \text{germination day};$$

and the flowering age as:

flowering age = flowering day – germination day .

The dry biomass was collected during six sequential, destructive harvests. Plant parts were separated for weighing into roots, rosette leaves and inflorescence (when there was one). Plants were dried at 80°C for three days and weighed to the nearest microgram. The focus was on the active stages of plant growth (mostly the vegetative phase) by harvesting at relevant points of the plants' development. Each harvest represents a developmental stage observed in most of the plants (Paul-Victor in prep).

The first harvest took place 7 days after sowing (DAS) when most plants had only two leaves. The second harvest took place 11 DAS when most plants had four leaves. The third harvest took place 15 DAS when most plants had six leaves. The fourth harvest took place 20 DAS when the plants started to bolt and had on average eight leaves. The fifth harvest took place 28 DAS when the first flowers were seen. The sixth harvest took place 33 DAS when the first fruits appeared. Even at the last harvest no siliques were observed to have opened and hence no biomass was lost as seeds. However, the rosettes were observed to have partially senesced. The number of leaves of each plant for all harvests was also counted.

Information about the 32 lines selected for the study. The two accessions L*er* and Cvi are the parents. The 30 remaining recombinant inbred lines are derived from reciprocal crosses between the two parents, see Appendix table 1.

NASC	RIL Koornneef	Published Seed Mass (*) [mg]	Sown Seed mass (**) [mg]	*ERECTA* mutation
N8581	L*er*	0.0193	0.0202	1
N8580	Cvi	0.0351	0.0348	0
N22002	CVL3	0.0162	0.0129	1
N22014	CVL15	0.0145	0.0193	0
N22018	CVL19	0.0251	0.0263	1
N22026	CVL27	0.0275	0.0270	1
N22030	CVL31	0.0295	0.0334	0
N22033	CVL34	0.0236	0.0297	0
N22036	CVL37	0.0325	0.0399	0
N22037	CVL38	0.0150	0.0188	0
N22038	CVL39	0.0202	0.0258	0
N22043	CVL44	0.0242	0.0285	0
N22051	CVL53	0.0327	0.0310	1
N22057	CVL60	0.0286	0.0393	1
N22059	CVL62	0.0190	0.0224	0
N22094	CVL124	0.0274	0.0252	1
N22095	CVL125	0.0200	0.0214	0
N22098	CVL128	0.0273	0.0274	0
N22099	CVL129	0.0243	0.0268	0
N22105	CVL135	0.0327	0.0348	1
N22107	CVL137	0.0302	0.0314	0
N22109	CVL139	0.0217	0.0231	0
N22112	CVL142	0.0315	0.0318	1
N22124	CVL154	0.0317	0.0323	0
N22128	CVL158	0.0373	0.0411	1
N22130	CVL160	0.0361	0.0402	1
N22132	CVL162	0.0256	0.0221	1
N22138	CVL168	0.0334	0.0299	0
N22148	CVL178	0.0207	0.0226	1
N22149	CVL179	0.0223	0.0243	1
N22156	CVL187	0.0183	0.0192	1
N22160	CVL191	0.0280	0.0257	1

(*) Source: Alonso-Blanco et al., 1999.
(**) Source: Arabidopsis center (TAIR).

Appendix table 1. The 30 remaining recombinant inbred lines.

Sand Dune Annuals: MATERIALS METHODS

Refer to: Turnbull et al.(2008)

The growth experiment

1724 individuals were grown, belonging to nine common sand-dune annual species from seed. The species (all nomenclature follows (Stace 1997)) and their mean seed masses are *Saxifraga tridactylites* (0.006 mg), *Erophila verna* (0.025 mg), *Cerastium diffusum* (0.045 mg), *Arenaria serpyllifolia* (0.088 mg), *Veronica arvensis* (0.112 mg), *Myosotis discolour* (0.213 mg), *Valerianella locusta* (0.851 mg), *Geranium molle* (1.094 mg) and *Erodium cicutarium* (2.92 mg). Although competitive interactions between these species have been intensively studied (e.g. Mack and Harper 1977; Rees et al. 1996; Coomes et al. 2002; Turnbull et al. 2004), little is currently known about their growth characteristics. Plants were grown in individual cells and watered regularly with one of five different dilutions of a complete nutrient solution. Above- and belowground parts were regularly harvested from September 2003 to April 2004 (a total of seven harvests). All plants were initially outside in an experimental garden; however, after five weeks, half of the plants were brought inside to a cool glasshouse where they were protected from frost damage. Daily temperature records were obtained for plants both inside and outside. Hours of daylight on each day of the experiment were calculated using the formula presented in Forsythe et al. (1985). From harvest number 4 onwards, we also took non-destructive measures (height and diameter) of all harvested plants. Using the resulting regression model between the destructive and non-destructive measures, the predicted biomass of unharvested plants was then estimated from which the same

non-destructive measures were taken; although this data was treated differently to that collected directly from destructive sampling.

The species (nomenclature follows Stace (1997)) and their mean seed masses are *Saxifraga tridactylites* (0.006 mg), *Erophila verna* (0.025 mg), *Cerastium diffusum* (0.045 mg), *Arenaria serpyllifolia* (0.088 mg), *Veronica arvensis* (0.112 mg), *Myosotis discolour* (0.213 mg), *Valerianella locusta* (0.851 mg), *Geranium molle* (1.094 mg), and *Erodium cicutarium* (2.92 mg). Seeds were allowed to germinate at their natural germination time (September 2003) and plants were grown until they set seed in May–June 2004. To mimic the typical small gaps in which these species are usually found, plants were grown singly in small cells measuring 13 × 13 mm and filled with sand plus a small amount of sieved peat and lime (in the proportions 6:1:1) to a depth of 26 mm. Cells were sown with more than one seed and thinned as soon as seedlings emerged to leave one seedling per cell (usually the first seedling to emerge). For the first five weeks following sowing, all cells were outside within glass-topped slug-proof enclosures in the University of Zurich experimental garden (47° 23' N, 8° 33' E, and 546 m a.s.l.). Fourteen days after sowing, 88% of the final plants had germinated and 15 individuals of each species were harvested, separated into above- and belowground parts, oven-dried and weighed. Following this initial harvest, each cell received one of five nutrient regimes (N) by applying either water (N = 0) or a complete nutrient solution in one of four dilutions (N = 0.25, 0.5, 0.75, 1) every two weeks. Plants were otherwise watered daily.

At the end of five weeks, temperatures began to fall close to zero and following a second destructive harvest, half of the remaining plants were brought inside to a cool greenhouse while the rest remained outside to experience winter temperatures. Plants inside the greenhouse received no additional lighting. The greenhouse had no

fine temperature control but temperatures were maintained between +5 and +20 degrees, so that plants would not experience extremes of either hot or cold. Harvests were approximately every 30 days, where possible, three individuals of each species from each nutrient regime both inside and outside (a total of seven destructive harvests) with the final one taking place in April (189 days after sowing). Three individuals from each species and nutrient regime both inside and outside were then left to finish setting seed. This scheme required a total of 1890 plants, of which 1724 (91%) were available. The "missing" plants were more or less equally distributed across species, treatments, and harvests. From the beginning of December (harvest number 4) height, number of leaves and/or rosette diameter was measured for all of the harvested and non-harvested plants. Following snowfall at the end of December, the glass covers were removed from the plants outside and they experienced ambient conditions, including snow cover, until the end of the experiment.

Daily minimum and maximum temperatures were obtained from a weather station within 1 km of the experimental garden (courtesy of MeteoSchweiz). In addition, weekly minimum and maximum temperatures were recorded inside the greenhouse. These fluctuated between a nighttime minimum of +3°C and a daytime maximum of +26 °C during the period November 2003 to April 2004 (compared to a nighttime minimum of –8.9 °C and a day-time maximum of +20.5 °C during the same period outside). For the initial period there were no thermometers under the glass covers. Daily minimum and maximum temperatures were measured under the same glass covers and the corresponding temperatures outside during a period when outside temperatures were similar to those during the growth experiment. By establishing regression relationships between the absolute temperature difference (outside vs. under glass) and the temperatures outside we were able to estimate temperatures

under the glass. Hours of daylight on each day of the experiment were calculated using the latitude of Zurich and the formula presented in Forsythe et al. (1985), which is accurate to between 1–7 minutes per day (Forsythe et al. 1985).

One of the drawbacks of multiple-harvest experiments is that very large numbers of plants need to be grown, although each plant is only harvested once. However, there is potential to increase the sample size at each harvest if the biomass of unharvested plants can be estimated from nondestructive measures (height, diameter, and/or number of leaves; McGraw and Garbutt 1990). Clearly this is only valid if the non-destructive measures can usefully predict plant-to-plant variation in biomass within experimental treatments. To assess this, a full model was fitted to the biomass data from each species at all harvest dates containing the terms harvest date, nutrient treatment and temperature regime (inside or outside) and all possible interactions. Once this full model was fit, additional terms were fitted, height, number of leaves, and/or rosette diameter. For all species' aboveground biomass, one or all of these nondestructive measures was highly significant, indicating that the non-destructive measures are an informative predictor of biomass variation within treatments. This model was then used to predict the biomass of unharvested plants; although these data were treated differently from those collected directly from destructive sampling.

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